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(54) Title: VECTORS HAVING BOTH ISOFORMS OF β-HEXOSAMINIDASE

(57) Abstract: Disclosed are compositions and methods related to nucleic acid constructs containing a HexB encoding element and a HexA encoding element. These constructs can be used in the treatment of Tray-Sachs and Sandoff disease.

VECTORS HAVING BOTH ISOFORMS OF β-HEXOSAMINIDASE

I.   ACKNOWLEDGMENTS

[01]   This application claims priority to United States Provisional Application No.
60/377,503 filed on May 2, 2003 for Vectors Having Both Isoforms of β-hexosamidase.
This application is herein incorporated by reference in its entirety.

II.   BACKGROUND OF THE INVENTION

[02]   Lysosomal storage disorders are disorders that typically arise from the
aberrant or non-existent proteins involved in degradation function within the lysosomes.
This causes a decrease in the lysosomal activity, which in turn causes an accumulation of
unwanted materials in the cell. These unwanted materials can cause severe cellular toxicity
and can impair, for example, neuronal function. These diseases severely impair the quality
of life of those who have them, and can even result in death. Two diseases, Tay-Sachs and
Sandoffs, are related to the functional impairment of the lysosomal protein β-
hexosaminidase. β-hexosaminidase is a hetero or homo dimer made up of two subunits
arising from two separate genes, HexA and HexB. Mutation of the HexA gene, causing
functional problems with the HEX-α (HexA/HexB) polypeptide, results in Tay-Sachs
disease, whereas mutation of the HexB gene, causing functional problems in the HEX-α
(HexA/HexB) and HEX-β (HexB/HexB) polypeptides, results in Sandhoff's disease.
Clinically, it is not uncommon for patients to display only mild features at infancy, but due
to increasing lysosomal storage over time, progress to severe forms of the disease by
adolescence.

[03]   Current treatments include bone marrow transplantation, which has been
employed in some cases of individuals during childhood but with modest outcomes. A
significant problem with the bone marrow transplantation approach is that it may address
the lack of specific metabolic activity in peripheral tissues, but due to the presence of the
blood-brain-barrier it fails to avert disease progression in the central nervous system. Hence
patients often continue to clinically deteriorate due to central nervous system involvement
with subsequent development of neurodegeneration, blindness, mental retardation, paralysis
and dementia.

[04]   Enzyme replacement strategies targeting peripheral and central nervous
system tissues utilizing gene therapy is a logical approach for treating inherited metabolic
disorders. In a study by Akli et al. (1996), the authors report successful restoration of β-

hexosaminidase in fibroblasts derived from patients with *HexA* deficiency via adenoviral-mediated gene transfer *in vitro*. Likewise a *HexA* transgene and a *HexB* transgene was successfully introduced into neural progenitor cells utilizing retroviral vectors (Lacorazza *et al.*, "Expression of human beta-hexosaminidase alpha-subunit gene (the gene defect of Tay-Sachs disease) in mouse brains upon engraftment of transduced progenitor cells".Nat Med 2(4):424-9 (1996 Apr).

[05]   Disclosed herein are vectors and methods which solve the problems associated with enzyme replacement therapies directed to β-hexosaminidase deficiencies.

III.   SUMMARY OF THE INVENTION

[06]   In accordance with the purposes of this invention, as embodied and broadly described herein, this invention, in one aspect, relates to vector constructs that comprise sequence encoding the HEX-β polypeptide. Also disclosed are vector constructs comprising sequence encoding the HEX-β and the HEX-α polypeptides. Also disclosed are vectors for perinatal gene delivery, including delivery of HEX-α and HEX-β, which can be used for inherited lysosomal disorders such as Tay-Sachs and Sandoffs disease.

[07]   Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

IV.   BRIEF DESCRIPTION OF THE DRAWINGS

[08]   The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention and together with the description, serve to explain the principles of the invention.

[09]   Figure 1 shows that HEX*lacZ* encodes for both isoforms of human β-hexosaminidase, HexA & HexB. Figure 1(A) shows *p*HEX*lacZ* vector. BHK[HexlacZ] are developed by stable *HexlacZ* transduction. Figure 3(B) shows cells stain positively by X-gal histochemistry. Figure 3(C) shows HexA & HexB mRNA is detected by RT-PCR in total RNA extracts. Figure 3(D₁) shows human HEXA & figure 3(E₁) shows human HEXB proteins are detected in BHK[HexlacZ] by imunocytochemistry. Figure 3(F₁) shows HEXA &

HEXA+HEXB activity is measured by 4MUGS & 4MUG fluorometry, respectively. Figure (G) β-hexosaminidase detection by Fast Garnet histochemistry. (D_2,E_2, G_2 are controls for D_1,E_1,G_1, respectively).

[10]   Figure 2 shows that the β-Hex therapeutic gene cross-corrects. An important property of the β-Hex transgene is the products hHEXA & hHEXB have the ability to cross-correct, specifically, to be released extracellularly and then to be absorbed via paracrine pathways by other cells whereby they contribute to β-hexosaminidase activity. For this purpose, $BHK^{HexlacZ}$ cells were cultured and the supernatant was collected (conditioned medium), filtered (.45mm) and applied on normal mouse kidney fibroblasts in culture. Forty-eight hours later, the cells were washed thoroughly with phosphate buffered saline, and briefly treated with a trypsin solution to remove extracellular proteins from the cell surfaces. Following trypsin inactivation with Tris/EDTA buffer, the cells were fixed with 4% paraformaldehyde solution and processed by Fast Garnet histochemistry for β-hexosaminidase activity. Fast Garnet histochemistry of murine fibroblasts exposed to (A) conditioned medium collected from $BHK^{HexlacZ}$ cells compared to cells exposed to medium from normal parent BHK-21 cells (B). These results demonstrate that hHEXA & hHEXB, products of the β-Hex transgene, are released into the extracellular medium and can be absorbed by other cells via paracrine pathways resulting in induction of the cellular β-hexosaminidase.

[11]   Figure 3 shows a representation of a lentiviral system containing the HexA and HexB genes. The 3-vector FIV(Hex) system The FIV(Hex) lentiviral system is comprised of 3 vectors: Packaging vector providing the packaging instructions in trans,- VSV-G envelop vector providing the envelop instructions in trans, - FIV(Hex) vector containing the therapeutic bicistronic gene.

[12]   Figure 4 shows a representation of a Fiv(Hex) vector. Backbone FIV vector constructed by Proeschla et al. (1998)

[13]   Figure 5 shows restriction fragment pattern of Feline immunodeficiency viral vector comprising a β-Hex construct. A maxi prep of FIV(Hex) clone 6.2 in 500 TB with 3X solution run through 2 columns. Yield of DNA was 1.095 mg. Final concentration is 1microg/microl. Restriction enzyme digest with ScaI, notI, SalI, and XhoI. The bands are as expected.

[14]   Figure 6 shows fibroblast infection by FIV(Hex) in vitro.

[15]   Figure 7 shows an FIV(Hex) titration experiment.

[16]   Figure 8 shows FIV(lacZ) administration to adult mice. FIV(lacZ) infection of murine fibroblasts (CrfK's) *in vitro*, as well as of liver cells following direct transdermal intra-hepatic injection. Liver, brain and spleen sections stained for β-galactosidase following intraperitoneal injection to 3 month old mice. lacZ expression was detected by X-gal staining (blue stain) and immunocytochemistry (ICC; black stain) on fixed tissue sections harvested 1 month post-treatment.

[17]   Figure 9 shows FIV(lacZ) administration to P4 mice. Liver, brain, spleen and kidney sections stained for β-galactosidase following intraperitoneal injection to mice of perinatal age (4 days old). lacZ expression was detected by X-gal staining (blue stain) on fixed tissue sections harvested 3 months post-treatment.

[18]   Figure 10 shows dose response of IP injections. Young adult mice (6 weeks old) were injected intra-peritoneally with different doses of FIV(lacZ) {0.1 mL, 0.5 mL, 1.0 mL and 2.0mL of 10^3 infectious particles per mL} viral solution. One month following treatment the animals were sacrificed and lacZ reporter gene expression was measured. It was found that increasing doses of FIV result in increasing levels of gene therapy efficacy. In the clinical, human disease arena, this would optimally translate into intravenous administration of 10^5-10^6 infectious FIV particles to ensure similar efficacy levels of gene therapy.

[19]   Figure 11 shows diagrams of the vectors used to make the constructs discussed in Examples 1 and 2. FIV(Hex) is constructed by ligating the backbone part of FIV(LacZ), and the fragment of HexB-IRES-HexA from pHexLacZ. FIV(LacZ) is 12750 bp, after cut with SstII and NotI (generate 4500 bp and 8250 bp bands). Purify the 8250 bp band which contains the FIV backbone with CMV promoter. pHexlacZ is a construct of 10150 bp. Cut with NheI and NotI, there are 4700 bp and 5450 bp fragments. The 4700 bp band contains the structure of HexB-IRES-HexA, which doesn't have CMV.

[20]   Figure 12 shows how the structure of FIV(Hex) was confirmed. The constructs were digested with different restriction enzymes: (Result see Figure 5). ScaI: cut once in the FIV backbone (generated one band 13 Kb). NotI: the site of ligation, and it is the only site (generated one band 13 Kb). Sal I: one site in HexB-IRFS-HexA and 3 sites in FIV backbone (generated one band t 8.5 Kb, one wide band with 2184 bp and 2400 bp, one band 34 bp which is invisible). Xho I: there is one site in HexB-IRFS-HexA and six

sites in the FIV backbone (FIV(LacZ): at 502, 1410, 1453, 7559, 7883 and 9949 bp). These generated 6 bands (908 bp, 43 bp(invisible), 1.7Kb, 324 bp, 2066 bp, 3.3 Kb, and 2.8 Kb).

[21]   Figure 13 shows a transcription termination cassette (STOP) flanked by 2 loxP sites was inserted between the promoter CMV and the therapeutic gene HexB-IRES-HexA. This results in inhibition of gene expression, until the STOP cassette is exsionally removed via the action of cre recombinase. The termination stop can consist of for example, a neomycin gene, whose termination signal acts as a termination signal for the rest of the transcript. Any reporter gene could be inserted and used in this way.

[22]   Figure 14 shows a dually regulated inducible cre-recombinase system which was constructed. The activity of this construct is regulated exogenously by RU486. Furthermore, a stable cell line for this system was developed, whereby addition of RU486 in the culture media results in activation of cre-recombinase and subsequently excisional recombination of DNA, such as a transcription termination cassette flanked by 2 loxP sites.

[23]   Figure 15 shows an example of the function of stable cell line, named GLVP/CrePr cell line, described in figure 14. In this case, the dual reporter vector CMV-lox-Luc-lox-AP was transiently transfected into the cell line. Alkaline phospatase (AP) activity was evaluated in vitro after the addition of RU486 to the culture media by an AP histochemical staining method.

[24]   Figure 16A shows the excisionally activated β-hexosaminidase gene Hex^{XAT} was constructed by placing a floxed transcription termination cassette (STOP) upstream to the first open reading frame: CMV-loxP-STOP-loxP-HexB-IRES-HexA. Figure 16B shows Hex^{XAT} was transiently transfected into our inducible cre cell line. Activation of cre-recombinase resulted in loxP directed DNA recombination and excision of the STOP cassette. Figure 16C Cre-mediated activation of Hex^{XAT} resulted in HexA and HexB upregulation (column 1). RU486 stimulation of GLVP/CrePr results in site-directed recombination and subsequent activation of a dormant transcriptional unit. A. shows the p Hex^{XAT}, a bicistronic transgene comprised of a "floxed" transcription-termination cassette (STOP), and both isoforms of the human β-hexosaminidase, was transiently trnasfected into the GLVP/CrePr cell line. B. RU-486 administration resulted in loxP-directed excisional recombination, C. resulting in transcriptional activation and synthesis of HexA and HexB mRNA.

[25]   Figure 17 shows the semi-quantitative analysis for HexA and HexB showed induction of gene transcription following Hex^{XAT} activation at (A) the mRNA level, (B) enzyme activity level in vitro, as well as (C) histochemical level in situ. RU486 significantly induces β-hexosaminidase expression in the GLVP/CrePr cell line. β-

5   hexosaminidase activity was found significantly upregulated in p Hex^{XAT} -transfected GLVP/CrePr cells 4 days after RU486 administration at the (A) HexA & HexB mRNA, (B) enzyme activity in vitro, as well as (C) in fixed monolayers in situ, as assessed by RTPCR, 4-MUG fluorescence and X-Hex histochemistry, respectively.

[26]   Figure 18 shows Hex^{XAT} was stably expressed in fibroblasts derived from a

10   patient with Tay-Sachs disease (TSD). Gene activation was mediated by infection of the cells with a HSV aplicon viral vector capable of transducing cells with the cre recombinase. This figure demonstrates that activation of the Hex gene results in protection of the TSD cells from death following GM$_2$ substrate challenge.

[27]   Figure 19 shows that the virus produced in Figure 3 above can resolve GM2

15   storage in TSD cells cultured in vitro.

[28]   Figure 20 shows the Hex gene was cloned in the FIV backbone as shown in Fig.3 producing the virus FIV(Hex), which was then used to infect TSD cells challenged with GM$_2$ substrate. This figures shows that delivery of our Hex gene with FIV(Hex) in TSD cells in vitro confers protection to cell death following GM$_2$ administration.

20   [29]   Figure 21 shows HexB$^{-/-}$ knock out pups (2 days) were injected 100uL of FIV(Hex) virus intraperitoneally. The animals were monitored weekly while they assumed growth until sacrificed (16-18 weeks of age).

[30]   Figure 22 shows expression of HEXB protein in adult mice that were injected with the FIV(Hex) virus as infants 2 days after birth. HEXB protein expression was

25   detected by immunocytochemistry in the liver and brain of these mice.

[31]   Figure 23 shows locomotive performance in relation to age (in weeks) of 6 mice that were treated 2 days after birth: 3 mice were injected with FIV(Hex) and 3 with FIV(lacZ) and served as controls. At 16 weeks of age, the "classic" stage that the hexB knockout mice display the disease, there was significant disease difference between the two

30   groups.

V.   DETAILED DESCRIPTION

[32]   The present invention may be understood more readily by reference to the following detailed description of preferred embodiments of the invention and the Examples included therein and to the Figures and their previous and following description.

5   [33]   Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that this invention is not limited to specific synthetic methods, specific recombinant biotechnology methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose

10   of describing particular embodiments only and is not intended to be limiting.

[34]   Disclosed are the components to be used to prepare the disclosed compositions as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that

15   while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular β-Hex vector is disclosed and discussed and a number of modifications that can be made to a number of molecules including the β-Hex vector are discussed, specifically contemplated is each and every

20   combination and permutation of the β-Hex vector and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E,

25   and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these

30   additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

A. Definitions

[35]   As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

[36]   Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It also understood that for every value disclosed, "about" that value is also disclosed. For example, if the value "10" is disclosed, then "about 10" is also disclosed, even if not specifically recited out as "about 10."

[37]   In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings:

[38]   "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

[39]   "Primers" are a subset of probes which are capable of supporting some type of enzymatic manipulation and which can hybridize with a target nucleic acid such that the enzymatic manipulation can occur. A primer can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art which do not interfere with the enzymatic manipulation.

[40]   "Probes" are molecules capable of interacting with a target nucleic acid, typically in a sequence specific manner, for example through hybridization. The hybridization of nucleic acids is well understood in the art and discussed herein. Typically a probe can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art.

[41]   Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into

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this application in order to more fully describe the state of the art to which this invention pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

B. Compositions and methods

1. Lysosomal disorders

[42] Lysosomal storage disorders are a group of closely related metabolic diseases resulting from deficiency in enzymes essential for the degradation of gangliosides, mucopolysaccharides, as well as other complex macromolecules. With the dysfunction of a lysosomal enzyme, catabolism of correlate substrates remains incomplete, leading to accumulation of insoluble complex macromolecules within the lysosomes. For example, β-hexosaminidase defects result in lysosomal storage of GM_2 gangliosides leading to the development of Tay-Sachs or Sandhoff's disease. Similarly, mucopolysaccharidoses (MPS) are a group of closely related metabolic disorders that result from deficiencies in lysosomal enzymes involved in glycosaminoglycan metabolism, leading to lysosomal mucopolysaccharide storage. Affected patients, depending on the specific disorder and clinical severity, may present with neurodegeneration, mental retardation, paralysis, dementia and blindness, dysostosis multiplex, craniofacial malformations and facial dysfiguration. Below, some of the most common conditions of this family of diseases are summarized.

Representative examples of common lysosomal storage disorders

Disease	Enzyme Deficiency	Storage Metabolite
Glycogenosis-Type 2	α-1,4-Glucosidase	Glycogen
Gangliosidoses GM_1 Gangliosidosis *Tay-Sachs disease* *Sandhoff disease*	GM_1 ganglioside β-galactosidase *Hexosaminidase – α subunit* *Hexosaminidase – β subunit*	GM_1 ganglioside *GM_2 ganglioside* *GM_2 ganglioside*
Sulfatidoses Krabbe disease Fabry disease Gaucher disease Niemann-Pick – types A & B	Galactosylceramidase α-Galactosidase A Glucocerebrosidase Sphingomyelinase	galactocerebroside ceramide trihexoside glucocerebroside sphingomyelin
Mucopolysaccharidoses Hurler's syndrome Hunter's syndrome	α-L-Iduronidase L-Iduronosulfate sulfatase	dermatan/heparan sulfate
Mucolipidoses Mucolipidosis – II Pseudo-Hurler's	Mannose-6-phosphate kinases	mucopolysaccharide/ glycolipid

Fucosidosis	α-Fucosidase	Glycoproteins
Mannosidosis	a-Mannosidase	oligosaccharides
Wolman Disease	Acid Lipase	triglycerides

2. Histopathology & Pathophysiology - A progressive disorder

[43]   In storage diseases, the affected cells become distended and display
vacuolated cytoplasms, which appear as swollen lysosomes under the electronic
microscope. For example, in the central nervous system, the neurons of the brain, trigeminal
and spinal root ganglia in patients suffering from GM_2 gangliodisoses display swollen
vacuolated perikarya stored with excessive amounts of lysosomal storage. As a result, these
organelles become large in size and numbers, interfering with normal cell functions. The
formation of meganeurites, axon hillock enlargements accompanied by secondary neuritic
sprouting, present as cardinal histopathological feature of gangliosidoses and
mucopolysaccharidoses (Purpura and Suzuki, 1976; Walkley et al., 1988). Purpura and
Suzuki proposed that meganeurites, and the synapses they develop, contribute to the onset
and progression of neuronal dysfunction in storage diseases, by altering electrical properties
of neurons and modifying integrative operations of somatodendritic synaptic inputs. In
addition, Walkley et al. (1991) suggested that this neuroaxonal dystrophy commonly
involved GABAergic neurons, and proposed that the resulting defect in neurotransmission
in inhibitory circuits may be an important factor underlying brain dysfunction in lysosomal
storage diseases. Consequently, the clinical phenotype often includes neurodegeneration,
mental retardation, paralysis, dementia and blindness. In addition, some storage disorders
also affect peripheral tissues, such as cartilage and bone, resulting in abnormal growth &
development of long bones, vertebrae, ribs and jaws, ultimately leading to anomalies of the
skeleton, the cranium and dysfiguration of the face (Mucopolysaccharidoses, and
Sandhoff's disease to some degree).

[44]   One cardinal characteristic of storage disorders is their progressively
worsening (progressive) nature. The deficiency of metabolic enzymes results in
accumulation of insoluble metabolites in the lysosomes, which becomes excessive and
deleterious over time due to the additive effects of accumulating insoluble metabolite
storage. For example, patients suffering from mucopolysaccharidoses (Hurler's or Hunter's)
display only a mild degree of the disease's phenotype at infancy, but, due to increasing
storage over time, progress to severe forms by adolescence, often leading to death (Gorlin et
al., 1990). This provides a window of opportunity in mammalian development during

which the pathophysiological process of the disease can be attenuated by restoring
lysosomal enzymatic activity early enough in life to prevent the development of a "full-
blown" disease and, perhaps, to reverse its progression.

3. Tay-Sachs & Sandhoffs disorders

5   [45]   The lysosomal enzyme β-hexosaminidase is comprised of 2 subunits
(peptides), HEX-α and HEX-β, encoded by two distinct genes, HexA and HexB,
respectively. β-hexosaminidase exists in 3 isoforms (proteins), HEXA (α/β heterodimer),
HEXB (β/β homodimer) and HEXS (α/α homodimer). Mutation of the HexA gene, causing
functional problems with the HEX-α polypeptide in humans results in Tay Sachs disease,

10   whereas mutation of the HexB gene, causing functional problems in the β-Hex polypeptide,
in Sandhoff's disease. In Tay Sachs disease, HexA mutation results in loss of HEXA
isoform (α/β heterodimer), whereas in Sandhoff's disease, HexB mutation results in loss of
both HEXA (α/β heterodimer) and HexB (β/β homodimer) isoforms, leading to a more
severe clinical phenotype. Affected patients, depending on the clinical severity, may

15   present with neurodegeneration, mental and motor deteriotation, dysarthria, impaired
thermal sensitivity, blindness, as well as facial dysfiguration (doll-like and coarse facies).
Histopathologically, the cells of the brain (neurons and glia), spleen and cartilage appear
swollen with vaculolated/clear perikarya suggestive of lysosomal storage. Biochemical
analysis reveals a complete lack of β-hexosaminidase activity accompanied by lysosomal

20   accumulation of GM_2 gangliosides. As a result, the lysosomes become large in size and
numbers, significantly crippling normal cellular function. Clinically, it is not uncommon
for patients to display only mild features at infancy, but due to increasing storage over time,
progress to severe forms of the disease by adolescence (Gorlin *et al.*, 1990). Similarly, other
affected mammals, such as affected mice pups, display only mild anomalies at birth, but

25   quickly develop their distinct abnormal features (1 month of age).

4. Blood brain barrier formation

        [46]   The blood-brain barrier (BBB) is a structure unique to the central nervous
system and is the result of tight junctions between the brain endothelial cells (Goldstein et
al., 1986). Previous work (Risau et al., 1986) on the development of mouse BBB using large

30   protein molecules (horse radish peroxidase) suggested BBB formation during the late days
of embryonic life (E17 in mouse). Furthermore, BBB in the adult is not absolute, whereby
certain areas of the brain do not develop BBB and thus allow for free exchange of

molecules through them. These areas include the median eminence (hypothalamus), pituitary, choroids plexus, pineal gland, subfornical organ, organum vasculosum lamina terminalis and area posterma (Risau & Wolburg, 1990). This allows for the intrusion of FIV(Hex) virions into the brain matter through an incomplete BBB as well as through areas
5   lacking BBB during the first few days after birth as discussed in the examples herein. Disclosed herein a diffuse expression of lacZ throughout the brain of P4 mice injected with FIV(lacZ) versus periventricular only localization following "adult administration" was shown.

5. Immune system development

10   [47]   Specific immunity in vertebrates is dependent on the host's ability to generate a heterogeneous repertoire of antigen-binding structures that are displayed on the surface of lymphocytes. Immunologic competence arises early in mammalian development. Since the expression of β-Hex therapeutic gene in $hexA^{-/-}/hexB^{-/-}$ mice may be perceived as presentation of "non-self" antigens, one needs to consider the possibility of an immune
15   response against human HEXA and HEXB following gene therapy. In these terms, perinatal administration can offer a unique opportunity in gene therapy application. Specifically, numerous studies have documented that the human and mouse neonate is unable to mount satisfactory responses to various antigenic challenges, which in many instances is delayed well beyond infancy (Schroeder et al., 1995). Therefore, due to this "immature"
20   immunologic state of mice and humans early in their postnatal life, perinatal gene therapy is consistent with adequate "training" of the immune system to recognize HEXA and HEXB as "self" antigens circumventing any potential immunologic rejection. It is understood that the transtherapy can take place in an infant as well.

        [48]   Disclosed are nucleic acids comprising sequence encoding HEX-α and
25   sequence encoding HEX-β. Also disclosed are nucleic acids, wherein the nucleic acid further comprises an IRES sequence, wherein the nucleic acids express more than one IRES sequence, wherein the vectors express an IRES sequence after each Hex nucleics acid, wherein the nucleic acid further comprises a promoter sequence, wherein the nucleic acid further comprises a promoter sequence, wherein the HEX-β has at least 80% identity to the
30   sequence set forth in SEQ ID NO:3 and the HEX-α has at least 80% identity to the sequence set forth in SEQ ID NO:1, wherein the HEX-β has at least 85% identity to the sequence set forth in SEQ ID NO:3 and the HEX-α has at least 80% identity to the

sequence set forth in SEQ ID NO:1, wherein the HEX-β has at least 90% identity to the

sequence set forth in SEQ ID NO:3 and the HEX-α has at least 80% identity to the

sequence set forth in SEQ ID NO:1, wherein the HEX-β has at least 95% identity to the

sequence set forth in SEQ ID NO:3 and the HEX-α has at least 80% identity to the

5   sequence set forth in SEQ ID NO:1, wherein the HEX-β has the sequence set forth in SEQ

ID NO:3 and the HEX-α has the sequence set forth in SEQ ID NO:1, wherein the sequence

encoding the HEX-β is orientated 5' to the sequence encoding HEX-α, wherein the

sequence encoding the HEX-β is orientated 5' to the IRES sequence and the IRES sequence

is located 5' to the sequence encoding HEX-α, wherein the promoter is located 5' to the

10   sequence encoding the HEX-β and the sequence encoding the HEX-β is orientated 5' to the

IRES sequence and the IRES sequence is located 5' to the sequence encoding HEX-α.

[49]   Also disclosed are vectors comprising the disclosed nucleic acids. Also

disclosed are cells comprising the disclosed nucleic acids and vectors.

[50]   Also disclosed are non-human mammal comprising the disclosed nucleic

15   acids, vectors, and cells disclosed herein.

[51]   Also disclosed are methods of providing HEX-α in a cell comprising

transfecting the cell with the nucleic acids, also disclosed are methods of providing HEX-β

in a cell comprising transfecting the cell with the nucleic acids, also disclosed are method of

providing HEX-α and HEX-β in a cell comprising transfecting the cell with the nucleic acid

20   of claims 1-4.

[52]   Also disclosed are method of delivering the disclosed compositions, wherein

the transfection occurs in vitro or in vivo.

[53]   Disclosed are methods of making a transgenic organism comprising

administering the disclosed nucleic acids, vectors and/or cells.

25   [54]   Disclosed are methods of making a transgenic organism comprising

transfecting a lentiviral vector to the organism at during a perinatal stage of the organism's

development.

[55]   Also disclosed are methods of treating a subject having Tay Sachs disease

and/or Sandoff disease comprising administering any of the disclosed compounds and

30   compositions.

C. Compositions

1. β-Hexosaminidase transgene (β-Hex)

[56] The β-Hexosaminidase protein is a protein comprised of two subunits, one subunit is encoded by the HexA gene and a second subunit encoded by the gene HexB. The human HexA Exon 1 can be found 316 bp upstream of MstII site; chromosome 15qll-15qter. The human HexA gene can be found at human chromosomal region 15q23----q24. The human HexB gene can be found on chromosome 5, map 5q13..

[57] Disclosed are constructs capable of expressing both the HexA gene product and the HexB gene product, from a single construct. Any construct capable of expressing both the HexA and HexB gene products is referred to as a β-Hex construct herein. The β-Hex construct allows for synthesis of all β-hexosaminidase protein isoforms, HEXA (α/β heterodimer), HEXB (β/β homodimer) and HEXS (α/α homodimer). Disclosed are nucleic acid constructs comprising a cytomegalovirus (CMV) promoter-driven bicistronic gene (β-Hex) that encodes for both human HexA and HexB genes, which can lead to the synthesis of functional β-hexosaminidase isoenzymes.

[58] The β-Hex construct typically comprises four parts: 1) a promoter, 2) the HexA coding sequence, 3) the HexB coding sequence, and 4) an IRES sequence (integrated ribosomal entry site). These four parts can be integrated into any vector delivery system. In preferred embodiments, the orientation of the four parts is 5'-promoter-HexB-IRES-HexA-3'.

[59] The promoter can be any promoter, such as those discussed herein. It is understood as discussed herein that there are functional variants of the HexA and HexB which can be made. Furthermore, it is understood that that there are functional variants of the IRES element, for example as discussed herein. Typically the genes to be expressed are placed on either side of the IRES sequence.

[60] The IRES element is an internal ribosomal entry sequence which can be iosolated from the encephalomyocarditis crius (ECMV). This element allows multiple genes to be expressed and correctly translated when the genes are on the same construct. IRES sequences are discussed in for example, United States Patent No: 4,937,190 which is herein incorporated by reference at least for material related to IRES sequences and their use.

[61]   HexA and HexB cDNA can be obtained from the American Tissue Culture

Collection. (American Tissue Culture Collection, Manassas, VA 20110-2209; Hex-α:

ATCC# 57206; Hex-β ATCC# 57350) The IRES sequence can be obtained from a number

of sources including commercial sources, such as the pIRES expressing vector from

5   Clonetech (Clontech, Palo Alto CA 94303-4230).

[62]   Also disclosed are tricistronic constructs encoding for both isoforms of

human β-hexosaminidase, hHexA & hHexB, as well as the β-galactosidase reporter gene

(*lacZ*). ·

[63]   Global delivery of the disclosed constructs is also disclosed. Disclosed is a

10   pseudotyped feline immunodeficiency virus (FIV) for global β-Hex delivery. Stable

expression of the therapeutic gene aids prolonged restoration of the genetic anomaly

enhancing treatment efficacy and contributing to long-term therapeutic outcomes. The

backbone FIV system has been shown to effectively incorporate, due to its lentiviral

properties, the transgene of interest into the host's genome, allowing for stable gene

15   expression (Poeschla et al., 1998). Disclosed herein is stable expression of the reporter gene

lacZ for over 3 months in mice following perinatal systemic FIV(lacZ) administration.

[64]   A model system for the study of these vectors is a mouse that is knockout

mouse deficient in both HexA and HexB, since the $hexA^{-/-}/hexB^{-/-}$ mouse is characterized by

global disruption of the *hexA* and *hexB* genes. Gene disruption in this mouse is global, and

20   therefore, can be used as a model for global replacement. The timing of gene therapy is

important as it is closely related to the temporal development of the disorder. $HexA^{-/-}/hexB^{-/-}$ mice display mild phenotype aberrations at birth and quickly develop craniofacial

dysplasia by 4-5 weeks of age. Similarly, it is not uncommon for patients suffering from

this class of genetic disorders to display only mild degree of the disease at infancy, and to

25   progress to severe forms by adolescence.

2. Delivery of the compositions to cells

[65]   Delivery can be applied, in general, via local or systemic routes of

administration. Local administration includes virus injection directly into the region or

organ of interest, versus intravenous (*IV*) or intraperitoneal (*IP*) injections (systemic)

30   aiming at viral delivery to multiple sites and organs via the blood circulation. Previous

research on the effects of local administration demonstrated gene expression limited to the

site/organ of the injection, which did not extend to the rest of the body (Daly et al., 1999a;

Kordower et al., 1999). Furthermore, previous studies have demonstrated successful global gene transfer to multiple tissues and organs in rodents and primates following viral *IV* and *IP* injections (Daly et al., 1999b; Tamtal et al., 2001; McCormack et al., 2001; Lipschutz et al., 2001). Disclosed herein *IP* injection of FIV(lacZ) in mice of adult (3 months old) as well as of perinatal age (P4) resulted in global transfer and expression of the reporter gene lacZ in brain, liver, spleen and kidney. Also disclosed, the levels of expression achieved via *IP* injections were superior to those acquired following local administration directly into the liver.

[66]   There are a number of compositions and methods which can be used to deliver nucleic acids to cells, either in vitro or in vivo. These methods and compositions can largely be broken down into two classes: viral based delivery systems and non-viral based delivery systems. For example, the nucleic acids can be delivered through a number of direct delivery systems such as, electroporation, lipofection, calcium phosphate precipitation, plasmids, viral vectors, viral nucleic acids, phage nucleic acids, phages, cosmids, or via transfer of genetic material in cells or carriers such as cationic liposomes. Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., Science, 247, 1465-1468, (1990); and Wolff, J. A. Nature, 352, 815-818, (1991)Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modified to specifically function with large DNA molecules. Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier.

a)   Nucleic acid based delivery systems

[67]   Transfer vectors can be any nucleotide construction used to deliver genes into cells (e.g., a plasmid), or as part of a general strategy to deliver genes, e.g., as part of recombinant retrovirus or adenovirus (Ram et al. Cancer Res. 53:83-88, (1993)).

[68]   As used herein, plasmid or viral vectors are agents that transport the disclosed nucleic acids, such as the β-Hex construct into the cell without degradation and include a promoter yielding expression of the HexA and HexB encoding sequences in the cells into which it is delivered. In some embodiments the vectors for the β-Hex constructs are derived from either a virus, retrovirus, or lentivirus. Viral vectors can be, for example,

Adenovirus, Adeno-associated virus, Herpes virus, Vaccinia virus, Polio virus, AIDS virus, neuronal trophic virus, Sindbis and other RNA viruses, including these viruses with the HIV backbone, and lentiviruses. Also preferred are any viral families which share the properties of these viruses which make them suitable for use as vectors. Retroviruses include Murine

5   Maloney Leukemia virus, MMLV, and retroviruses that express the desirable properties of MMLV as a vector. Retroviral vectors are able to carry a larger genetic payload, i.e., a transgene, such as, the disclosed β-Hex constructs or marker gene, than other viral vectors, and for this reason are a commonly used vector. However, they are not as useful in non-proliferating cells. Adenovirus vectors are relatively stable and easy to work with, have

10   high titers, and can be delivered in aerosol formulation, and can transfect non-dividing cells. Pox viral vectors are large and have several sites for inserting genes, they are thermostable and can be stored at room temperature. A preferred embodiment is a viral vector, which has been engineered so as to suppress the immune response of the host organism, elicited by the viral antigens. Preferred vectors of this type will carry coding regions for Interleukin 8 or

15   10.

[69]   Viral vectors can have higher transaction (ability to introduce genes) abilities than chemical or physical methods to introduce genes into cells. Typically, viral vectors contain, nonstructural early genes, structural late genes, an RNA polymerase III transcript, inverted terminal repeats necessary for replication and encapsidation, and promoters to

20   control the transcription and replication of the viral genome. When engineered as vectors, viruses typically have one or more of the early genes removed and a gene or gene/promotor cassette is inserted into the viral genome in place of the removed viral DNA. Constructs of this type can carry up to about 8 kb of foreign genetic material. The necessary functions of the removed early genes are typically supplied by cell lines which have been engineered to

25   express the gene products of the early genes in trans.

(1) Retroviral Vectors

[70]   A retrovirus is an animal virus belonging to the virus family of Retroviridae, including any types, subfamilies, genus, or tropisms. Retroviral vectors, in general, are described by Verma, I.M., Retroviral vectors for gene transfer. In Microbiology-1985,

30   American Society for Microbiology, pp. 229-232, Washington, (1985), which is incorporated by reference herein. Examples of methods for using retroviral vectors for gene therapy are described in U.S. Patent Nos. 4,868,116 and 4,980,286; PCT applications WO

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90/02806 and WO 89/07136; and Mulligan, (Science 260:926-932 (1993)); the teachings of which are incorporated herein by reference.

[71]   A retrovirus is essentially a package which has packed into it nucleic acid cargo. The nucleic acid cargo carries with it a packaging signal, which ensures that the replicated daughter molecules will be efficiently packaged within the package coat. In addition to the package signal, there are a number of molecules which are needed in cis, for the replication, and packaging of the replicated virus. Typically a retroviral genome, contains the gag, pol, and env genes which are involved in the making of the protein coat. It is the gag, pol, and env genes which are typically replaced by the foreign DNA that it is to be transferred to the target cell. Retrovirus vectors typically contain a packaging signal for incorporation into the package coat, a sequence which signals the start of the gag transcription unit, elements necessary for reverse transcription, including a primer binding site to bind the tRNA primer of reverse transcription, terminal repeat sequences that guide the switch of RNA strands during DNA synthesis, a purine rich sequence 5' to the 3' LTR that serve as the priming site for the synthesis of the second strand of DNA synthesis, and specific sequences near the ends of the LTRs that enable the insertion of the DNA state of the retrovirus to insert into the host genome. The removal of the gag, pol, and env genes allows for about 8 kb of foreign sequence to be inserted into the viral genome, become reverse transcribed, and upon replication be packaged into a new retroviral particle. This amount of nucleic acid is sufficient for the delivery of a one to many genes depending on the size of each transcript. It is preferable to include either positive or negative selectable markers along with other genes in the insert.

[72]   Since the replication machinery and packaging proteins in most retroviral vectors have been removed (gag, pol, and env), the vectors are typically generated by placing them into a packaging cell line. A packaging cell line is a cell line which has been transfected or transformed with a retrovirus that contains the replication and packaging machinery, but lacks any packaging signal. When the vector carrying the DNA of choice is transfected into these cell lines, the vector containing the gene of interest is replicated and packaged into new retroviral particles, by the machinery provided in cis by the helper cell. The genomes for the machinery are not packaged because they lack the necessary signals.

(2) Adenoviral Vectors

[73]   The construction of replication-defective adenoviruses has been described (Berkner et al., J. Virology 61:1213-1220 (1987); Massie et al., Mol. Cell. Biol. 6:2872-2883 (1986); Haj-Ahmad et al., J. Virology 57:267-274 (1986); Davidson et al., J. Virology 61:1226-1239 (1987); Zhang "Generation and identification of recombinant adenovirus by liposome-mediated transfection and PCR analysis" BioTechniques 15:868-872 (1993)). The benefit of the use of these viruses as vectors is that they are limited in the extent to which they can spread to other cell types, since they can replicate within an initial infected cell, but are unable to form new infectious viral particles. Recombinant adenoviruses have been shown to achieve high efficiency gene transfer after direct, in vivo delivery to airway epithelium, hepatocytes, vascular endothelium, CNS parenchyma and a number of other tissue sites (Morsy, J. Clin. Invest. 92:1580-1586 (1993); Kirshenbaum, J. Clin. Invest. 92:381-387 (1993); Roessler, J. Clin. Invest. 92:1085-1092 (1993); Moullier, Nature Genetics 4:154-159 (1993); La Salle, Science 259:988-990 (1993); Gomez-Foix, J. Biol. Chem. 267:25129-25134 (1992); Rich, Human Gene Therapy 4:461-476 (1993); Zabner, Nature Genetics 6:75-83 (1994); Guzman, Circulation Research 73:1201-1207 (1993); Bout, Human Gene Therapy 5:3-10 (1994); Zabner, Cell 75:207-216 (1993); Caillaud, Eur. J. Neuroscience 5:1287-1291 (1993); and Ragot, J. Gen. Virology 74:501-507 (1993)). Recombinant adenoviruses achieve gene transduction by binding to specific cell surface receptors, after which the virus is internalized by receptor-mediated endocytosis, in the same manner as wild type or replication-defective adenovirus (Chardonnet and Dales, Virology 40:462-477 (1970); Brown and Burlingham, J. Virology 12:386-396 (1973); Svensson and Persson, J. Virology 55:442-449 (1985); Seth, et al., J. Virol. 51:650-655 (1984); Seth, et al., Mol. Cell. Biol. 4:1528-1533 (1984); Varga et al., J. Virology 65:6061-6070 (1991); Wickham et al., Cell 73:309-319 (1993)).

[74]   A viral vector can be one based on an adenovirus which has had the E1 gene removed and these virons are generated in a cell line such as the human 293 cell line. In another preferred embodiment both the E1 and E3 genes are removed from the adenovirus genome.

(3) Adeno-asscociated viral vectors

[75]   Another type of viral vector is based on an adeno-associated virus (AAV). This defective parvovirus is a preferred vector because it can infect many cell types and is nonpathogenic to humans. AAV type vectors can transport about 4 to 5 kb and wild type

AAV is known to stably insert into chromosome 19. Vectors which contain this site specific integration property are preferred. An especially preferred embodiment of this type of vector is the P4.1 C vector produced by Avigen, San Francisco, CA, which can contain the herpes simplex virus thymidine kinase gene, HSV-tk, and/or a marker gene, such as the gene encoding the green fluorescent protein, GFP.

[76]   In another type of AAV virus, the AAV contains a pair of inverted terminal repeats (ITRs) which flank at least one cassette containing a promoter which directs cell-specific expression operably linked to a heterologous gene. Heterologous in this context refers to any nucleotide sequence or gene which is not native to the AAV or B19 parvovirus.

[77]   Typically the AAV and B19 coding regions have been deleted, resulting in a safe, noncytotoxic vector. The AAV ITRs, or modifications thereof, confer infectivity and site-specific integration, but not cytotoxicity, and the promoter directs cell-specific expression. United states Patent No. 6,261,834 is herein incorproated by reference for material related to the AAV vector.

[78]   The vectors of the present invention thus provide DNA molecules which are capable of integration into a mammalian chromosome without substantial toxicity.

[79]   The inserted genes in viral and retroviral usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and may contain upstream elements and response elements.

(4) Lentiviral vectors

[01]   The vectors can be lentiviral vectors, including but not limited to, SIV vectors, HIV vectors or a hybrid construct of these vectors, including viruses with the HIV backbone. These vectors also include first, second and third generation lentiviruses. Third generation lentiviruses have lentiviral packaging genes split into at least 3 independent plasmids or constructs. Also vectors can be any viral family that share the properties of these viruses which make them suitable for use as vectors. Lentiviral vectors are a special type of retroviral vector which are typically characterized by having a long incubation period for infection. Furthermore, lentiviral vectors can infect non-dividing cells.

Lentiviral vectors are based on the nucleic acid backbone of a virus from the lentiviral

family of viruses. Typically, a lentiviral vector contains the 5' and 3' LTR regions of a

lentivirus, such as SIV and HIV. Lentiviral vectors also typically contain the Rev

Responsive Element (RRE) of a lentivirus, such as SIV and HIV.

5   *(a) Feline immunodeficiency viral vectors*

[80]   One type of vector that the disclosed constructs can be delivered in is the

VSV-G pseudotyped Feline Immunodeficiency Virus system developed by Poeschla *et al.*

(1998). This lentivirus has been shown to efficiently infect dividing, growth arrested as well

as post-mitotic cells. Furthermore, due to its lentiviral properties, it allows for incorporation

10   of the transgene into the host's genome, leading to stable gene expression. This is a 3-vector

system, whereby each confers distinct instructions: the FIV vector carries the transgene of

interest and lentiviral apparatus with mutated packaging and envelope genes. A vesicular

stomatitis virus G-glycoprotein vector (VSV-G; Burns et al., 1993) contributes to the

formation of the viral envelope *in trans*. The third vector confers packaging instructions *in*

15   *trans* (Poeschla *et al.*, 1998). FIV production is accomplished *in vitro* following co-

transfection of the aforementioned vectors into 293-T cells. The FIV-rich supernatant is

then collected, filtered and can be used directly or following concentration by

centrifugation. Titers routinely range between $10^4 - 10^7$ bfu/ml..

                                              (5) Packaging vectors

20   [81]   As discussed above, retroviral vectors are based on retroviruses which

contain a number of different sequence elements that control things as diverse as integration

of the virus, replication of the integrated virus, replication of un-integrated virus, cellular

invasion, and packaging of the virus into infectious particles. While the vectors in theory

could contain all of their necessary elements, as well as an exogenous gene element (if the

25   exogenous gene element is small enough) typically many of the necessary elements are

removed. Since all of the packaging and replication components have been removed from

the typical retroviral, including lentiviral, vectors which will be used within a subject, the

vectors need to be packaged into the initial infectious particle through the use of packaging

vectors and packaging cell lines. Typically retroviral vectors have been engineered so that

30   the myriad functions of the retrovirus are separated onto at least two vectors, a packaging

vector and a delivery vector. This type of system then requires the presence of all of the

vectors providing all of the elements in the same cell before an infectious particle can be

produced. The packaging vector typically carries the structural and replication genes

derived from the retrovirus, and the delivery vector is the vector that carries the exogenous gene element that is preferably expressed in the target cell. These types of systems can split the packaging functions of the packaging vector into multiple vectors, e.g., third-generation lentivirus systems. Dull, T. et al., "A Third-generation lentivirus vector with a conditional packaging system"J. Virol 72(11):8463-71 (1998)

[82]   Retroviruses typically contain an envelope protein (env). The Env protein is in essence the protein which surrounds the nucleic acid cargo. Furthermore cellular infection specificity is based on the particular Env protein associated with a typical retrovirus. In typical packaging vector/delivery vector systems, the Env protein is expressed from a separate vector than for example the protease (pro) or integrase (in) proteins.

(6) Packaging cell lines

[83]   The vectors are typically generated by placing them into a packaging cell line. A packaging cell line is a cell line which has been transfected or transformed with a retrovirus that contains the replication and packaging machinery, but lacks any packaging signal. When the vector carrying the DNA of choice is transfected into these cell lines, the vector containing the gene of interest is replicated and packaged into new retroviral particles, by the machinery provided in cis by the helper cell. The genomes for the machinery are not packaged because they lack the necessary signals. One type of packaging cell line is a 293 cell line.

(7) Large payload viral vectors

[84]   Molecular genetic experiments with large human herpesviruses have provided a means whereby large heterologous DNA fragments can be cloned, propagated and established in cells permissive for infection with herpesviruses (Sun et al., Nature genetics 8: 33-41, 1994; Cotter and Robertson,.Curr Opin Mol Ther 5: 633-644, 1999). These large DNA viruses (herpes simplex virus (HSV) and Epstein-Barr virus (EBV), have the potential to deliver fragments of human heterologous DNA > 150 kb to specific cells. EBV recombinants can maintain large pieces of DNA in the infected B-cells as episomal DNA. Individual clones carried human genomic inserts up to 330 kb appeared genetically stable The maintenance of these episomes requires a specific EBV nuclear protein, EBNA1, constitutively expressed during infection with EBV. Additionally, these vectors can be used for transfection, where large amounts of protein can be generated transiently in vitro.

Herpesvirus amplicon systems are also being used to package pieces of DNA > 220 kb and to infect cells that can stably maintain DNA as episomes.

[85]   Other useful systems include, for example, replicating and host-restricted non-replicating vaccinia virus vectors.

b) Non-nucleic acid based systems

[86]   The disclosed compositions can be delivered to the target cells in a variety of ways. For example, the compositions can be delivered through electroporation, or through lipofection, or through calcium phosphate precipitation. The delivery mechanism chosen will depend in part on the type of cell targeted and whether the delivery is occurring for example in vivo or in vitro.

[87]   Thus, the compositions can comprise, in addition to the disclosed constructs or vectors for example, lipids such as liposomes, such as cationic liposomes (e.g., DOTMA, DOPE, DC-cholesterol) or anionic liposomes. Liposomes can further comprise proteins to facilitate targeting a particular cell, if desired. Administration of a composition comprising a compound and a cationic liposome can be administered to the blood afferent to a target organ or inhaled into the respiratory tract to target cells of the respiratory tract. Regarding liposomes, see, e.g., Brigham et al. *Am. J. Resp. Cell. Mol. Biol.* 1:95-100 (1989); Felgner et al. *Proc. Natl. Acad. Sci USA* 84:7413-7417 (1987); U.S. Pat. No.4,897,355. Furthermore, the compound can be administered as a component of a microcapsule that can be targeted to specific cell types, such as macrophages, or where the diffusion of the compound or delivery of the compound from the microcapsule is designed for a specific rate or dosage.

[88]   In the methods described above which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), delivery of the compositions to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, MD), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, WI), as well as other liposomes developed according to procedures standard in the art. In addition, the nucleic acid or vector of this invention can be delivered *in vivo* by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, AZ).

[89]   The materials may be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate

5   Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). These techniques can be used for a variety of other speciifc cell

10   types. Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells in vivo. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al.,

15   Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored

20   intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency,

25   and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

[90]   Nucleic acids that are delivered to cells which are to be integrated into the host cell genome, typically contain integration sequences. These sequences are often viral

30   related sequences, particularly when viral based systems are used. These viral intergration systems can also be incorporated into nucleic acids which are to be delivered using a non-nucleic acid based system of deliver, such as a liposome, so that the nucleic acid contained in the delivery system can be come integrated into the host genome.

[91]   Other general techniques for integration into the host genome include, for

example, systems designed to promote homologous recombination with the host genome.

These systems typically rely on sequence flanking the nucleic acid to be expressed that has

enough homology with a target sequence within the host cell genome that recombination

5   between the vector nucleic acid and the target nucleic acid takes place, causing the

delivered nucleic acid to be integrated into the host genome. These systems and the

methods necessary to promote homologous recombination are known to those of skill in the

art.

c) In vivo/ex vivo

10   [92]   As described herein, the compositions can be administered in a

pharmaceutically acceptable carrier and can be delivered to the subjects cells *in vivo* and/or

ex vivo by a variety of mechanisms well known in the art (e.g., uptake of naked DNA,

liposome fusion, intramuscular injection of DNA via a gene gun, endocytosis and the like).

[93]   If *ex vivo* methods are employed, cells or tissues can be removed and

15   maintained outside the body according to standard protocols well known in the art. The

compositions can be introduced into the cells via any gene transfer mechanism, such as, for

example, calcium phosphate mediated gene delivery, electroporation, microinjection or

proteoliposomes. The transduced cells can then be infused (e.g., in a pharmaceutically

acceptable carrier) or homotopically transplanted back into the subject per standard

20   methods for the cell or tissue type. Standard methods are known for transplantation or

infusion of various cells into a subject.

[94]   If in vivo delivery methods are performed the methods can be designed to

deliver the nucleic acid constructs directly to a particular cell type, via any delivery

mechanism, such as intra-peritoneal injection of a vector construct. In this type of delivery

25   situation, the nucleic acid constructs can be delivered to any type of tissue, for example,

brain or neural or muscle. The nucleic acid constructs can also be delivered such that they

generally deliver the nucleic acid constructs to more than one type of cell. This type of

delivery can be accomplished, by for example, injecting the constructs intraperitoneally into

the flank of the organism. (See Example 2 and figures 8-10). It in certain delivery

30   methods, the timing of the delivery is monitored. For example, the nucleic acid constructs

can be delivered at the perinatal stage of the recipients life or at the adult stage.

[95]   The disclosed compositions, can be delivered to any type of cell. For example, they can be delivered to any type of mammalian cell. Exemplary types of cells neuron, glia, fibroblast, chondrocyte, osteocyte, endothelial, and hepatocyte.

3.   Expression systems

5   [96]   The nucleic acids that are delivered to cells typically contain expression controlling systems. For example, the inserted genes in viral and retroviral systems usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core

10   elements required for basic interaction of RNA polymerase and transcription factors, and may contain upstream elements and response elements.

a)   Viral Promoters and Enhancers

[97]   Preferred promoters controlling transcription from vectors in mammalian host cells may be obtained from various sources, for example, the genomes of viruses such

15   as: polyoma, Simian Virus 40 (SV40), adenovirus, retroviruses, hepatitis-B virus and most preferably cytomegalovirus, or from heterologous mammalian promoters, e.g. beta actin promoter. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature, 273: 113 (1978)). The immediate early promoter of the human

20   cytomegalovirus is conveniently obtained as a HindIII E restriction fragment (Greenway, P.J. et al., Gene 18: 355-360 (1982)). Of course, promoters from the host cell or related species also are useful herein.

[98]   Enhancer generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' (Laimins, L. et al., Proc. Natl.

25   Acad. Sci. 78: 993 (1981)) or 3' (Lusky, M.L., et al., Mol. Cell Bio. 3: 1108 (1983)) to the transcription unit. Furthermore, enhancers can be within an intron (Banerji, J.L. et al., Cell 33: 729 (1983)) as well as within the coding sequence itself (Osborne, T.F., et al., Mol. Cell Bio. 4: 1293 (1984)). They are usually between 10 and 300 bp in length, and they function in cis. Enhancers f unction to increase transcription from nearby promoters.

30   Enhancers also often contain response elements that mediate the regulation of transcription. Promoters can also contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression of a gene. While many enhancer

sequences are now known from mammalian genes (globin, elastase, albumin, -fetoprotein and insulin), typically one will use an enhancer from a eukaryotic cell virus for general expression. Preferred examples are the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

[99] The promoter and/or enhancer may be specifically activated either by light or specific chemical events which trigger their function. Systems can be regulated by reagents such as tetracycline and dexamethasone. There are also ways to enhance viral vector gene expression by exposure to irradiation, such as gamma irradiation, or alkylating chemotherapy drugs.

[100] In certain embodiments the promoter and/or enhancer region can act as a constitutive promoter and/or enhancer to maximize expression of the region of the transcription unit to be transcribed. In certain constructs the promoter and/or enhancer region be active in all eukaryotic cell types, even if it is only expressed in a particular type of cell at a particular time. A preferred promoter of this type is the CMV promoter (650 bases). Other preferred promoters are SV40 promoters, cytomegalovirus (full length promoter), and retroviral vector LTF.

[101] It has been shown that all specific regulatory elements can be cloned and used to construct expression vectors that are selectively expressed in specific cell types such as melanoma cells. The glial fibrillary acetic protein (GFAP) promoter has been used to selectively express genes in cells of glial origin.

[102] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) may also contain sequences necessary for the termination of transcription which may affect mRNA expression. These regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3' untranslated regions also include transcription termination sites. It is preferred that the transcription unit also contain a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA. The identification and use of polyadenylation signals in expression constructs is well established. It is preferred that homologous polyadenylation signals be used in the transgene constructs. In certain transcription units, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists

of about 400 bases. It is also preferred that the transcribed units contain other standard

sequences alone or in combination with the above sequences improve expression from, or

stability of, the construct.

[103] In certain embodiments the promoters are constitutive promoters. This can

5   be any promoter that causes transcription regulation in the absence of the addition of other

factors. Examples of this type of promoter are the CMV promoter and the beta actin

promoter, as well as others dicussed herein. In certain embodiments the promoter can

consist of fusions of one or more different types of promoters. For example, the regulatory

regions of the CMV promoter and the beta actin promoter are well known and understood,

10 examples, of which are disclosed herein. Parts of these promoters can be fused together to,

for example, produce a CMV-beta actin fusion promoter, such as the one shown in SEQ ID

NO:23. It is understood that this type of promoter has a CMV component and a beta actin

component. These components can function independently as promoters, and thus, are

themselves considered beta actin promoters and CMV promoters. A promoter can be any

15 portion of a known promoter that causes promoter activity. It is well understood that many

promoters, including the CMV and Beta Actin promoters have functional domains which

are understood and that these can be used as a beta actin promoter or CMV promoter.

Furthermore, these domains can be determined. For example, SEQ ID NO:s 21-41 display

a number of CMV promoters, beta actin promoters, and fusion promoters. These promoters

20 can be compared, and for example, functional regions delineated, as described herein.

Furthermore, each of these sequences can function independently or together in any

combination to provide a promoter region for the disclosed nucleic acids.

b) Markers

[104] The viral vectors can include nucleic acid sequence encoding a marker

25 product. This marker product is used to determine if the gene has been delivered to the cell

and once delivered is being expressed. Preferred marker genes are the *E. Coli* lacZ gene,

which encodes ß-galactosidase, and green fluorescent protein.

[105] In some embodiments the marker may be a selectable marker. Examples of

suitable selectable markers for mammalian cells are dihydrofolate reductase (DHFR),

30 thymidine kinase, neomycin, neomycin analog G418, hydromycin, and puromycin. When

such selectable markers are successfully transferred into a mammalian host cell, the

transformed mammalian host cell can survive if placed under selective pressure. There are

two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to grow independent of a supplemented media. Two examples are: CHO DHFR- cells and mouse LTK- cells. These cells lack the ability to grow without the addition of such nutrients as

5   thymidine or hypoxanthine. Because these cells lack certain genes necessary for a complete nucleotide synthesis pathway, they cannot survive unless the missing nucleotides are provided in a supplemented media. An alternative to supplementing the media is to introduce an intact DHFR or TK gene into cells lacking the respective genes, thus altering their growth requirements. Individual cells which were not transformed with the DHFR or

10   TK gene will not be capable of survival in non-supplemented media.

[106] The second category is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These schemes typically use a drug to arrest growth of a host cell. Those cells which have a novel gene would express a protein conveying drug resistance and would survive the selection.

15   Examples of such dominant selection use the drugs neomycin, (Southern P. and Berg, P., J. Molec. Appl. Genet. 1: 327 (1982)), mycophenolic acid, (Mulligan, R.C. and Berg, P. Science 209: 1422 (1980)) or hygromycin, (Sugden, B. et al., Mol. Cell. Biol. 5: 410-413 (1985)). The three examples employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid)

20   or hygromycin, respectively. Others include the neomycin analog G418 and puramycin.

c) Post transcriptional regulatory elements

[107] The disclosed vectors can also contain post-transcriptional regulatory elements. Post-transcriptional regulatory elements can enhance mRNA stability or enhance translation of the transcribed mRNA. An exemplary post-transcriptional regulatory

25   sequence is the WPRE sequence isolated from the woodchuck hepatitis virus. (Zufferey R, et al., "Woodchuck hepatitis virus post-transcriptional regulatory element enhances expression of transgenes delivered by retroviral vectors," J Virol; 73:2886-92 (1999)). Post-transcriptional regulatory elements can be positioned both 3' and 5' to the exogenous gene, but it is preferred that they are positioned 3' to the exogenous gene.

30   ### d) Transduction efficiency elements

[108] Transduction efficiency elements are sequences that enhance the packaging and transduction of the vector. These elements typically contain polypurine sequences. An

example of a transduction efficiency element is the ppt-cts sequence that contains the central polypurine tract (ppt) and central terminal site (cts) from the HIV-1 pSG3 molecular clone (SEQ ID. NO:1 bp 4327 to 4483 of HIV-1 pSG3 clone).

e) 3' untranslated regions

[109] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) may also contain sequences necessary for the termination of transcription which may affect mRNA expression. These 3' untranslated regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding the exogenous gene. The 3' untranslated regions also include transcription termination sites. The transcription unit also can contain a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA. The identification and use of polyadenylation signals in expression constructs is well established. Homologous polyadenylation signals can be used in the transgene constructs. In an embodiment of the transcription unit, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists of about 400 bases. Transcribed units can contain other standard sequences alone or in combination with the above sequences improve expression from, or stability of, the construct.

4. Sequence similarities

[110] It is understood that as discussed herein the use of the terms homology and identity mean the same thing as similarity. Thus, for example, if the use of the word homology is used between two non-natural sequences it is understood that this is not necessarily indicating an evolutionary relationship between these two sequences, but rather is looking at the similarity or relatedness between their nucleic acid sequences. Many of the methods for determining homology between two evolutionarily related molecules are routinely applied to any two or more nucleic acids or proteins for the purpose of measuring sequence similarity regardless of whether they are evolutionarily related or not.

[111] In general, it is understood that one way to define any known variants and derivatives or those that might arise, of the disclosed genes and proteins herein, is through defining the variants and derivatives in terms of homology to specific known sequences. This identity of particular sequences disclosed herein is also discussed elsewhere herein. In general, variants of genes and proteins herein disclosed typically have at least, about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95,

96, 97, 98, or 99 percent homology to the stated sequence or the native sequence. Those of
skill in the art readily understand how to determine the homology of two proteins or nucleic
acids, such as genes. For example, the homology can be calculated after aligning the two
sequences so that the homology is at its highest level.

5   [112] Another way of calculating homology can be performed by published
algorithms. Optimal alignment of sequences for comparison may be conducted by the local
homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the
homology alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443 (1970), by
the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85:

10   2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT,
FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer
Group, 575 Science Dr., Madison, WI), or by inspection.

           [113] The same types of homology can be obtained for nucleic acids by for
example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc.*

15   *Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306,
1989 which are herein incorporated by reference for at least material related to nucleic acid
alignment. It is understood that any of the methods typically can be used and that in certain
instances the results of these various methods may differ, but the skilled artisan understands
if identity is found with at least one of these methods, the sequences would be said to have

20   the stated identity, and be disclosed herein.

           [114] For example, as used herein, a sequence recited as having a particular
percent homology to another sequence refers to sequences that have the recited homology
as calculated by any one or more of the calculation methods described above. For example,
a first sequence has 80 percent homology, as defined herein, to a second sequence if the

25   first sequence is calculated to have 80 percent homology to the second sequence using the
Zuker calculation method even if the first sequence does not have 80 percent homology to
the second sequence as calculated by any of the other calculation methods. As another
example, a first sequence has 80 percent homology, as defined herein, to a second sequence
if the first sequence is calculated to have 80 percent homology to the second sequence using

30   both the Zuker calculation method and the Pearson and Lipman calculation method even if
the first sequence does not have 80 percent homology to the second sequence as calculated
by the Smith and Waterman calculation method, the Needleman and Wunsch calculation
method, the Jaeger calculation methods, or any of the other calculation methods. As yet

another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using each of calculation methods (although, in practice, the different calculation methods will often result in different calculated homology percentages).

5   **5. Hybridization/selective hybridization**

[115]   The term hybridization typically means a sequence driven interaction between at least two nucleic acid molecules, such as a primer or a probe and a gene. Sequence driven interaction means an interaction that occurs between two nucleotides or nucleotide analogs or nucleotide derivatives in a nucleotide specific manner. For example,

10   G interacting with C or A interacting with T are sequence driven interactions. Typically sequence driven interactions occur on the Watson-Crick face or Hoogsteen face of the nucleotide. The hybridization of two nucleic acids is affected by a number of conditions and parameters known to those of skill in the art. For example, the salt concentrations, pH, and temperature of the reaction all affect whether two nucleic acid molecules will hybridize.

15

[116]   Parameters for selective hybridization between two nucleic acid molecules are well known to those of skill in the art. For example, in some embodiments selective hybridization conditions can be defined as stringent hybridization conditions. For example, stringency of hybridization is controlled by both temperature and salt concentration of

20   either or both of the hybridization and washing steps. For example, the conditions of hybridization to achieve selective hybridization may involve hybridization in high ionic strength solution (6X SSC or 6X SSPE) at a temperature that is about 12-25°C below the Tm (the melting temperature at which half of the molecules dissociate from their hybridization partners) followed by washing at a combination of temperature and salt

25   concentration chosen so that the washing temperature is about 5°C to 20°C below the Tm. The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to a labeled nucleic acid of interest and then washed under conditions of different stringencies. Hybridization temperatures are typically higher for DNA-RNA and RNA-RNA

30   hybridizations. The conditions can be used as described above to achieve stringency, or as is known in the art. (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989; Kunkel et al. Methods Enzymol. 1987:154:367, 1987 which is herein incorporated by reference for

material at least related to hybridization of nucleic acids). A preferable stringent
hybridization condition for a DNA:DNA hybridization can be at about 68°C (in aqueous
solution) in 6X SSC or 6X SSPE followed by washing at 68°C. Stringency of hybridization
and washing, if desired, can be reduced accordingly as the degree of complementarity

5   desired is decreased, and further, depending upon the G-C or A-T richness of any area
wherein variability is searched for. Likewise, stringency of hybridization and washing, if
desired, can be increased accordingly as homology desired is increased, and further,
depending upon the G-C or A-T richness of any area wherein high homology is desired, all
as known in the art.

10   [117] Another way to define selective hybridization is by looking at the amount
(percentage) of one of the nucleic acids bound to the other nucleic acid. For example, in
some embodiments selective hybridization conditions would be when at least about, 60, 65,
70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93,
94, 95, 96, 97, 98, 99, 100 percent of the limiting nucleic acid is bound to the non-limiting

15   nucleic acid. Typically, the non-limiting primer is in for example, 10 or 100 or 1000 fold
excess. This type of assay can be performed at under conditions where both the limiting
and non-limiting primer are for example, 10 fold or 100 fold or 1000 fold below their k_d, or
where only one of the nucleic acid molecules is 10 fold or 100 fold or 1000 fold or where
one or both nucleic acid molecules are above their k_d.

20   [118] Another way to define selective hybridization is by looking at the percentage
of primer that gets enzymatically manipulated under conditions where hybridization is
required to promote the desired enzymatic manipulation. For example, in some
embodiments selective hybridization conditions would be when at least about, 60, 65, 70,
71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94,

25   95, 96, 97, 98, 99, 100 percent of the primer is enzymatically manipulated under conditions
which promote the enzymatic manipulation, for example if the enzymatic manipulation is
DNA extension, then selective hybridization conditions would be when at least about 60,
65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92,
93, 94, 95, 96, 97, 98, 99, 100 percent of the primer molecules are extended. Preferred

30   conditions also include those suggested by the manufacturer or indicated in the art as being
appropriate for the enzyme performing the manipulation.

        [119] Just as with homology, it is understood that there are a variety of methods
herein disclosed for determining the level of hybridization between two nucleic acid

molecules. It is understood that these methods and conditions may provide different percentages of hybridization between two nucleic acid molecules, but unless otherwise indicated meeting the parameters of any of the methods would be sufficient. For example if 80% hybridization was required and as long as hybridization occurs within the required

5   parameters in any one of these methods it is considered disclosed herein.

[120]   It is understood that those of skill in the art understand that if a composition or method meets any one of these criteria for determining hybridization either collectively or singly it is a composition or method that is disclosed herein.

6. Nucleic acids

10   [121]   There are a variety of molecules disclosed herein that are nucleic acid based, including for example the nucleic acids that encode, for example HexA and HexB, or functional nucleic acids. The disclosed nucleic acids can be made up of for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for example, when a vector

15   is expressed in a cell, that the expressed mRNA will typically be made up of A, C, G, and U. Likewise, it is understood that if, for example, an antisense molecule is introduced into a cell or cell environment through for example exogenous delivery, it is advantagous that the antisense molecule be made up of nucleotide analogs that reduce the degradation of the antisense molecule in the cellular environment.

20   [122]   A nucleotide is a molecule that contains a base moiety, a sugar moiety and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and sugar moieties creating an internucleoside linkage. The base moiety of a nucleotide can be adenin-9-yl (A), cytosin-1-yl (C), guanin-9-yl (G), uracil-1-yl (U), and thymin-1-yl (T). The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a

25   nucleotide is pentavalent phosphate. An non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate).

[123]   A nucleotide analog is a nucleotide which contains some type of modification to either the base, sugar, or phosphate moieties. Modifications to nucleotides are well known in the art and would include for example, 5-methylcytosine (5-me-C),

30   5-hydroxymethyl cytosine, xanthine, hypoxanthine, and 2-aminoadenine as well as modifications at the sugar or phosphate moieties.

[124] Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a

5   phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

[125] It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are

10   not limited to lipid moieties such as a cholesterol moiety. (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989,86, 6553-6556),

[126] A Watson-Crick interaction is at least one interaction with the Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6

15   positions of a purine based nucleotide, nucleotide analog, or nucleotide substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

[127] A Hoogsteen interaction is the interaction that takes place on the Hoogsteen face of a nucleotide or nucleotide analog, which is exposed in the major groove of duplex

20   DNA. The Hoogsteen face includes the N7 position and reactive groups (NH2 or O) at the C6 position of purine nucleotides.

a) Sequences

[128] There are a variety of sequences related to the HexA, HexB, IRES sequences, and promoter sequences. For example, the HexA and hexB genes have the

25   following Genbank Accession Numbers: M16411and NM_000520 for HexA and NM_000521 for HexB, these sequences and others are herein incorporated by reference in their entireties as well as for individual subsequences contained therein. It is understood that there are numerous Genbank accession sequences related to HexA and HexB, all of which are incorporated by reference herein.

30   [129] One particular sequence set forth in SEQ ID NO:4 and having Genbank accession number NM_000521, which is a sequence for human HexB cDNA, is used herein, as an example, to exemplify the disclosed compositions and methods. It is

understood that the description related to this sequence is applicable to any sequence related to HexA or HexB unless specifically indicated otherwise. Those of skill in the art understand how to resolve sequence discrepancies and differences and to adjust the compositions and methods relating to a particular sequence to other related sequences.

5   Primers and/or probes can be designed for any of the sequences disclosed herein given the information disclosed herein and that known in the art.

[130] It is also understood for example that there are numerous bicistronic vectors that can be used to create the β-Hex construct nucleic acids See for example, Genbank accession no Y11035 and Y11034.

10   **b) Primers and probes**

[131] Disclosed are compositions including primers and probes, which are capable of interacting with, for example, the β-Hex construct nucleic acids, as disclosed herein. In certain embodiments the primers are used to support DNA amplification reactions. Typically the primers will be capable of being extended in a sequence specific manner.

15   Extension of a primer in a sequence specific manner includes any methods wherein the sequence and/or composition of the nucleic acid molecule to which the primer is hybridized or otherwise associated directs or influences the composition or sequence of the product produced by the extension of the primer. Extension of the primer in a sequence specific manner therefore includes, but is not limited to, PCR, DNA sequencing, DNA extension,

20   DNA polymerization, RNA transcription, or reverse transcription. Techniques and conditions that amplify the primer in a sequence specific manner are preferred. In certain embodiments the primers are used for the DNA amplification reactions, such as PCR or direct sequencing. It is understood that in certain embodiments the primers can also be extended using non-enzymatic techniques, where for example, the nucleotides or

25   oligonucleotides used to extend the primer are modified such that they will chemically react to extend the primer in a sequence specific manner. Typically the disclosed primers hybridize with, for example, the β-Hex construct nucleic acid, or region of the β-Hex construct nucleic acids or they hybridize with the complement of the β-Hex construct nucleic acids or complement of a region of the β-Hex construct nucleic acids.

7. Peptides

a) Protein variants

[132] As discussed herein there are numerous variants of the HEX-α and HEX-β
proteins that are known and herein contemplated. In addition, to the known functional
species and allelic variants of HEX-α and HEX-β there are derivatives of the HEX-α and
HEX-β proteins which also function in the disclosed methods and compositions. Protein
variants and derivatives are well understood to those of skill in the art and in can involve
amino acid sequence modifications. For example, amino acid sequence modifications
typically fall into one or more of three classes: substitutional, insertional or deletional
variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence
insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller
insertions than those of amino or carboxyl terminal fusions, for example, on the order of
one to four residues. Immunogenic fusion protein derivatives, such as those described in
the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity
to the target sequence by cross-linking in vitro or by recombinant cell culture transformed
with DNA encoding the fusion. Deletions are characterized by the removal of one or more
amino acid residues from the protein sequence. Typically, no more than about from 2 to 6
residues are deleted at any one site within the protein molecule. These variants ordinarily
are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein,
thereby producing DNA encoding the variant, and thereafter expressing the DNA in
recombinant cell culture. Techniques for making substitution mutations at predetermined
sites in DNA having a known sequence are well known, for example M13 primer
mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single
residues, but can occur at a number of different locations at once; insertions usually will be
on the order of about from 1 to 10 amino acid residues; and deletions will range about from
1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a
deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any
combination thereof may be combined to arrive at a final construct. The mutations must not
place the sequence out of reading frame and preferably will not create complementary
regions that could produce secondary mRNA structure. Substitutional variants are those in
which at least one residue has been removed and a different residue inserted in its place.
Such substitutions generally are made in accordance with the following Tables 1 and 2 and
are referred to as conservative substitutions.

[133]   TABLE 1:Amino Acid Abbreviations

Amino Acid	Abbreviations
alanine	AlaA
allosoleucine	AIle
arginine	ArgR
asparagine	AsnN
aspartic acid	AspD
cysteine	CysC
glutamic acid	GluE
glutamine	GlnK
glycine	GlyG
histidine	HisH
isolelucine	IleI
leucine	LeuL
lysine	LysK
phenylalanine	PheF
proline	ProP
pyroglutamic acidp	Glu
serine	SerS
threonine	ThrT
tyrosine	TyrY
tryptophan	TrpW
valine	ValV

TABLE 2:Amino Acid Substitutions	
Original Residue	Exemplary Conservative Substitutions, others are known in the art.
Ala	ser
Arg	lys, gln
Asn	gln; his
Asp	glu
Cys	ser
Gln	asn, lys
Glu	asp
Gly	pro
His	asn;gln
Ile	leu; val
Leu	ile; val
Lys	arg; gln;
Met	Leu; ile
Phe	met; leu; tyr
Ser	thr
Thr	ser
Trp	tyr
Tyr	trp; phe
Val	ile; leu

[134]   Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 2, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the

bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other

5　residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

10　[135] For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr;

15　Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the mosaic polypeptides provided herein.

[136] Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also may be desirable. Deletions or substitutions of potential

20　proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

[137] Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl

25　residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco pp 79-86 [1983]),

30　acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

[138] It is understood that one way to define the variants and derivatives of the disclosed proteins herein is through defining the variants and derivatives in terms of homology/identity to specific known sequences. For example, SEQ ID NO:1 sets forth a particular sequence of HEX-α and SEQ ID NO:3 sets forth a particular sequence of a HEX-

5   β protein. Specifically disclosed are variants of these and other proteins herein disclosed which have at least, 70% or 75% or 80% or 85% or 90% or 95% homology to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

10   [139] Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85:

15   2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

[140] The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc.*

20   *Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment.

[141] It is understood that the description of conservative mutations and homology can be combined together in any combination, such as embodiments that have at least 70%

25   homology to a particular sequence wherein the variants are conservative mutations.

[142] As this specification discusses various proteins and protein sequences it is understood that the nucleic acids that can encode those protein sequences are also disclosed. This would include all degenerate sequences related to a specific protein sequence, i.e. all nucleic acids having a sequence that encodes one particular protein sequence as well as all

30   nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed

and described herein through the disclosed protein sequence. For example, one of the many nucleic acid sequences that can encode the protein sequence set forth in SEQ ID NO:3 is set forth in SEQ ID NO:4. Another nucleic acid sequence that encodes the same protein sequence set forth in SEQ ID NO:3 is set forth in SEQ ID NO:11. In addition, for example,

5   a disclosed conservative derivative of SEQ ID NO:3 is shown in SEQ ID NO: 12, where the valine (V) at position 21 is changed to a isoleucine (I). It is understood that for this mutation all of the nucleic acid sequences that encode this particular derivative of the SEQ ID NO:3 polypeptide are also disclosed. It is also understood that while no amino acid sequence indicates what particular DNA sequence encodes that protein within an organism,

10   where particular variants of a disclosed protein are disclosed herein, the known nucleic acid sequence that encodes that protein in the particular organism from which that protein arises is also known and herein disclosed and described.

8.   Pharmaceutical carriers/Delivery of pharamceutical products

[143]   As described above, the compositions can also be administered *in vivo* in a

15   pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be

20   selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

[144]   The compositions may be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, topically or the like, including topical intranasal administration or

25   administration by inhalant. As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the nucleic acid or vector. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism.

30   Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the compositions required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the allergic disorder being treated, the particular nucleic acid or vector used, its

mode of administration and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

[145]   Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

[146]   The materials may be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand,

ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

a) Pharmaceutically Acceptable Carriers

5   [147]   The compositions, including antibodies, can be used therapeutically in combination with a pharmaceutically acceptable carrier.

[148]   Suitable carriers and their formulations are described in *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995. Typically, an appropriate amount of a pharmaceutically-acceptable salt is

10   used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the

15   antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of composition being administered.

[149]   Pharmaceutical carriers are known to those skilled in the art. These most

20   typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

[150]   Pharmaceutical compositions may include carriers, thickeners, diluents, buffers,

25   preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

[151]   The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated.

30   Administration may be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection. The disclosed antibodies can be administered

intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally.

[152]   Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are
5   propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers,
10   electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

[153]   Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers,
15   aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

[154]   Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable..

[155]   Some of the compositions may potentially be administered as a
20   pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium
25   hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

9.   Chips and micro arrays

[156]   Disclosed are chips where at least one address is the sequences or part of the sequences set forth in any of the nucleic acid sequences disclosed herein. Also disclosed
30   are chips where at least one address is the sequences or portion of sequences set forth in any of the peptide sequences disclosed herein.

[157] Also disclosed are chips where at least one address is a variant of the sequences or part of the sequences set forth in any of the nucleic acid sequences disclosed herein. Also disclosed are chips where at least one address is a variant of the sequences or portion of sequences set forth in any of the peptide sequences disclosed herein.

10. Computer readable mediums

[158] It is understood that the disclosed nucleic acids and proteins can be represented as a sequence consisting of the nucleotides of amino acids. There are a variety of ways to display these sequences, for example the nucleotide guanosine can be represented by G or g. Likewise the amino acid valine can be represented by Val or V. Those of skill in the art understand how to display and express any nucleic acid or protein sequence in any of the variety of ways that exist, each of which is considered herein disclosed. Specifically contemplated herein is the display of these sequences on computer readable mediums, such as, commercially available floppy disks, tapes, chips, hard drives, compact disks, and video disks, or other computer readable mediums. Also disclosed are the binary code representations of the disclosed sequences. Those of skill in the art understand what computer readable mediums. Thus, computer readable mediums on which the nucleic acids or protein sequences are recorded, stored, or saved.

[159] Disclosed are computer readable mediums comprising the sequences and information regarding the sequences set forth herein.

11. Kits

[160] Disclosed herein are kits that are drawn to reagents that can be used in practicing the methods disclosed herein. The kits can include any reagent or combination of reagent discussed herein or that would be understood to be required or beneficial in the practice of the disclosed methods. For example, the kits could include primers to perform the amplification reactions discussed in certain embodiments of the methods, as well as the buffers and enzymes required to use the primers as intended.

D. Methods of making the compositions

[161] The compositions disclosed herein and the compositions necessary to perform the disclosed methods can be made using any method known to those of skill in the art for that particular reagent or compound unless otherwise specifically noted.

[162] The disclosed viral vectors can be made using standard recombinant molecular biology techniques. Many of these techniques are illustrated in Maniatis

(Maniatis et al., *Molecular Cloning--A Laboratory Manual,*" (Cold Spring Harbor Laboratory, Latest edition) and Sambrook et al., *Molecular Cloning: A Laboratory Manual,* 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989.

1. Nucleic acid synthesis

5   [163]   For example, the nucleic acids, such as, the oligonucleotides to be used as primers can be made using standard chemical synthesis methods or can be produced using enzymatic methods or any other known method. Such methods can range from standard enzymatic digestion followed by nucleotide fragment isolation (see for example, Sambrook *et al., Molecular Cloning: A Laboratory Manual,* 2nd Edition (Cold Spring Harbor

10   Laboratory Press, Cold Spring Harbor, N.Y., 1989) Chapters 5, 6) to purely synthetic methods, for example, by the cyanoethyl phosphoramidite method using a Milligen or Beckman System Plus DNA synthesizer (for example, Model 8700 automated synthesizer of Milligen-Biosearch, Burlington, MA or ABI Model 380B). Synthetic methods useful for making oligonucleotides are also described by Ikuta *et al., Ann. Rev. Biochem.* 53:323-356

15   (1984), (phosphotriester and phosphite-triester methods), and Narang *et al., Methods Enzymol.,* 65:610-620 (1980), (phosphotriester method). Protein nucleic acid molecules can be made using known methods such as those described by Nielsen *et al., Bioconjug. Chem.* 5:3-7 (1994).

2. Peptide synthesis

20   [164]   One method of producing the disclosed proteins is to link two or more peptides or polypeptides together by protein chemistry techniques. For example, peptides or polypeptides can be chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (*tert* -butyloxycarbonoyl) chemistry. (Applied Biosystems, Inc., Foster City, CA). One skilled

25   in the art can readily appreciate that a peptide or polypeptide corresponding to the disclosed proteins, for example, can be synthesized by standard chemical reactions. For example, a peptide or polypeptide can be synthesized and not cleaved from its synthesis resin whereas the other fragment of a peptide or protein can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group which is functionally blocked on the other

30   fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, to form an antibody, or fragment thereof. (Grant GA (1992) Synthetic Peptides: A User Guide. W.H. Freeman and Co., N.Y. (1992); Bodansky M and Trost B., Ed. (1993) Principles of Peptide Synthesis.

Springer-Verlag Inc., NY (which is herein incorporated by reference at least for material

related to peptide synthesis). Alternatively, the peptide or polypeptide is independently

synthesized *in vivo* as described herein. Once isolated, these independent peptides or

polypeptides may be linked to form a peptide or fragment thereof via similar peptide

5   condensation reactions.

[165] For example, enzymatic ligation of cloned or synthetic peptide segments

allow relatively short peptide fragments to be joined to produce larger peptide fragments,

polypeptides or whole protein domains (Abrahmsen L et al., Biochemistry, 30:4151

(1991)). Alternatively, native chemical ligation of synthetic peptides can be utilized to

10   synthetically construct large peptides or polypeptides from shorter peptide fragments. This

method consists of a two step chemical reaction (Dawson et al. Synthesis of Proteins by

Native Chemical Ligation. Science, 266:776-779 (1994)). The first step is the

chemoselective reaction of an unprotected synthetic peptide--thioester with another

unprotected peptide segment containing an amino-terminal Cys residue to give a

15   thioester-linked intermediate as the initial covalent product. Without a change in the

reaction conditions, this intermediate undergoes spontaneous, rapid intramolecular reaction

to form a native peptide bond at the ligation site (Baggiolini M et al. (1992) FEBS Lett.

307:97-101; Clark-Lewis I et al., J.Biol.Chem., 269:16075 (1994); Clark-Lewis I et al.,

Biochemistry, 30:3128 (1991); Rajarathnam K et al., Biochemistry 33:6623-30 (1994)).

20   [166] Alternatively, unprotected peptide segments are chemically linked where the

bond formed between the peptide segments as a result of the chemical ligation is an

unnatural (non-peptide) bond (Schnolzer, M et al. Science, 256:221 (1992)). This technique

has been used to synthesize analogs of protein domains as well as large amounts of

relatively pure proteins with full biological activity (deLisle Milton RC et al., Techniques in

25   Protein Chemistry IV. Academic Press, New York, pp. 257-267 (1992)).

3. Processes for making the compositions

[167] Disclosed are processes for making the compositions as well as making the

intermediates leading to the compositions. There are a variety of methods that can be used

for making these compositions, such as synthetic chemical methods and standard molecular

30   biology methods. It is understood that the methods of making these and the other disclosed

compositions are specifically disclosed.

[168]   Disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a promoter element, a HexB element, a IRES element, and a HexA element.

[169]   Disclosed are nucleic acid molecules produced by the process comprising linking in an operative way nucleic acid molecules comprising sequences set forth in SEQ ID NO:10 and SEQ ID NO:4.

[170]   Also disclosed are nucleic acid molecules produced by the process comprising linking in an operative way nucleic acid molecules comprising sequences having 80% identity to sequences set forth in SEQ ID NO:10 and SEQ ID NO:4.

[171]   Also disclosed are nucleic acid molecules produced by the process comprising linking in an operative way nucleic acid molecules comprising sequences that hybridizes under stringent hybridization conditions to sequences set forth in SEQ ID NO:10 and SEQ ID NO:4.

[172]   Disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a nucleic acid molecule comprising a sequence encoding HEX-β and HEX-α peptides and a sequence controlling an expression of the sequence encoding HEX-β and HEX-α.

[173]   Disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a nucleic acid molecule comprising a sequence encoding HEX-β and HEX-α peptides wherein the HEX-β and HEX-α peptides have 80% identity to the peptides set forth in SEQ ID NO:1 and SEQ ID NO:3 and a sequence controlling expression of the sequences encoding the peptides.

[174]   Disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a nucleic acid molecule comprising a sequence encoding HEX-β and HEX-α peptides wherein the HEX-β and HEX-α peptides have 80% identity to the peptides set forth in SEQ ID NO:1 and SEQ ID NO:3, wherein any change from the sequences set forth in SEQ ID NO:1 and SEQ ID NO:3 are conservative changes and a sequence controlling expression of the sequences encoding the peptides.

[175]   Disclosed are cells produced by the process of transforming the cell with any of the disclosed nucleic acids. Disclosed are cells produced by the process of transforming the cell with any of the non-naturally occurring disclosed nucleic acids.

— 48 —

[176]   Disclosed are any of the disclosed peptides produced by the process of
expressing any of the disclosed nucleic acids. Disclosed are any of the non-naturally
occurring disclosed peptides produced by the process of expressing any of the disclosed
nucleic acids. Disclosed are any of the disclosed peptides produced by the process of
5   expressing any of the non-naturally disclosed nucleic acids.

[177]   Disclosed are animals produced by the process of transfecting a cell within
the animal with any of the nucleic acid molecules disclosed herein. Disclosed are animals
produced by the process of transfecting a cell within the animal any of the nucleic acid
molecules disclosed herein, wherein the animal is a mammal. Also disclosed are animals
10   produced by the process of transfecting a cell within the animal any of the nucleic acid
molecules disclosed herein, wherein the mammal is mouse, rat, rabbit, cow, sheep, pig, or
primate. Also disclosed are mammals wherein mammal is a murine, ungulate, or non-
human primate.

[178]   Also disclose are animals produced by the process of adding to the animal
15   any of the cells disclosed herein.

E. Methods of using the compositions

1. Methods of using the compositions as research tools

[179]   The disclosed compositions can be used in a variety of ways as research
tools. For example, the disclosed compositions, the ß-Hex constructs, and other nucleic
20   acids, such as SEQ ID NOs:10 and 4 can be used to produce organisms, such as transgenic
or knockout mice, which can be used as model systems for the study of Tay Sachs and
Sandoffs disease.

2. Methods of gene modification and gene disruption

[180]   The disclosed compositions and methods can be used for targeted gene
25   disruption and modification in any animal that can undergo these events. Gene
modification and gene disruption refer to the methods, techniques, and compositions that
surround the selective removal or alteration of a gene or stretch of chromosome in an
animal, such as a mammal, in a way that propagates the modification through the germ line
of the mammal. In general, a cell is transformed with a vector which is designed to
30   homologously recombine with a region of a particular chromosome contained within the
cell, as for example, described herein. This homologous recombination event can produce a
chromosome which has exogenous DNA introduced, for example in frame, with the

surrounding DNA. This type of protocol allows for very specific mutations, such as point
mutations, to be introduced into the genome contained within the cell. Methods for
performing this type of homologous recombination are disclosed herein.

[181] One of the preferred characteristics of performing homologous
recombination in mammalian cells is that the cells should be able to be cultured, because
the desired recombination event occurs at a low frequency.

[182] Once the cell is produced through the methods described herein, an animal
can be produced from this cell through either stem cell technology or cloning technology.
For example, if the cell into which the nucleic acid was transfected was a stem cell for the
organism, then this cell, after transfection and culturing, can be used to produce an
organism which will contain the gene modification or disruption in germ line cells, which
can then in turn be used to produce another animal that possesses the gene modification or
disruption in all of its cells. In other methods for production of an animal containing the
gene modification or disruption in all of its cells, cloning technologies can be used. These
technologies generally take the nucleus of the transfected cell and either through fusion or
replacement fuse the transfected nucleus with an oocyte which can then be manipulated to
produce an animal. The advantage of procedures that use cloning instead of ES technology
is that cells other than ES cells can be transfected. For example, a fibroblast cell, which is
very easy to culture can be used as the cell which is transfected and has a gene modification
or disruption event take place, and then cells derived from this cell can be used to clone a
whole animal.

3. Therapeutic Uses

[183] Effective dosages and schedules for administering the compositions may be
determined empirically, and making such determinations is within the skill in the art. The
dosage ranges for the administration of the compositions are those large enough to produce
the desired effect in which the symptoms disorder are effected. The dosage should not be
so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic
reactions, and the like. Generally, the dosage will vary with the age, condition, sex and
extent of the disease in the patient, route of administration, or whether other drugs are
included in the regimen, and can be determined by one of skill in the art. The dosage can be
adjusted by the individual physician in the event of any counterindications. Dosage can
vary, and can be administered in one or more dose administrations daily, for one or several

days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products.

[184] Following administration of a disclosed composition, such as the disclosed constructs, for treating, inhibiting, or preventing Tay Sachs or Sandoffs disease, the efficacy

5   of the therapeutic construct can be assessed in various ways well known to the skilled practitioner. For instance, one of ordinary skill in the art will understand that a composition, such as the disclosed constructs, disclosed herein is efficacious in treating Tay Sachs or Sandoffs disease or inhibiting or reducing the effects of Tay Sachs or Sandoffs disease in a subject by observing that the composition reduces the onset of the conditions

10   associated with these diseases. Furthermore, the amount of protein or transcript produced from the constructs can be analyzed using any diagnostic method. For example, it can be measured using polymerase chain reaction assays to detect the presence of construct nucleic acid or antibody assays to detect the presence of protein produced from the construct in a sample (e.g., but not limited to, blood or other cells, such as neural cells) from a subject or

15   patient.

F. Examples

[185] It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the

20   art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

[186] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions,

25   articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or

30   is at ambient temperature, and pressure is at or near atmospheric.

1. Example 1 Making β-Hex constructs

a) Construction of bicistronic β-Hex construct

[187]   A bicistronic construct encoding for both isoforms of human β-hexosaminidase, hHexA and hHexB was made (Figure 1). hHexB cDNA was isolated

5   following *Xho I* digestion of pHexB43 (ATCC, Manassas VA) and cloned into the *Xho* I site of pIRES (Clonetech Laboratories, Palo Alto CA) downstream of the vector's cytomegalovirus (CMV) promoter sequence. The HexA cDNA was isolated from pBHA-5 (ATCC, Manassas VA) by *Xho I* digestion and was subsequently inserted into the *Xba I* site of pIRES(HexB) downstream of the vectors IRES cassette by blunt ligation. In this

10   construct, the cytomegalovirus promoter (CMV) drives transgene expression, and the translation of the second open reading frame, *HexB,* is facilitated by an internal ribosomal entry sequence (IRES).

b) Results

[188]   The HEX*lacZ* encodes for both isoforms of human β-hexosaminidase, HexA

15   & HexB. (Figure 1) The vector *pHEXlacZ* is shown in Figure 1(A). BHK^{HexlacZ} are developed by stable *HexlacZ* transduction. Figure 1(B) shows that the cells transfected with the *p*HEX*lacZ* vector stain positively by X-gal histochemistry. Furthermore, HexA & HexB mRNA was detected by RT-PCR in total RNA extracts (Figure 1(C)). Likewise, not only was transcript of *pHEXlacZ* vector identified, human HEXA and human HEXB

20   proteins were detected in the transfected BHK^{HexlacZ} cells by imunocytochemistry. (Figure 1(D$_1$) and 1(E$_1$). This data indicates that the disclosed constructs can be expressed in target cells and that sufficient levels of protein are produced within these cells.

[189]   The β-Hex therapeutic gene is capable of correcting deficiencies in cells that are not transfected through cross-correction. (Figure 2) An important property of the β-

25   Hex transgene is the products hHEXA & hHEXB have the ability to cross-correct, specifically, to be released extracellularly and then to be absorbed via paracrine pathways by other cells whereby they contribute to β-hexosaminidase activity. BHK^{HexlacZ} cells were cultured and the supernatant was collected (conditioned medium), filtered (.45mm) and applied on normal mouse kidney fibroblasts in culture. Forty-eight hours later, the cells

30   were washed thoroughly with phosphate buffered saline, and briefly treated with a trypsin solution to remove extracellular proteins from the cell surfaces. Following trypsin inactivation with Tris/EDTA buffer, the cells were fixed with 4% paraformaldehyde

solution and processed by Fast Garnet histochemistry for β-hexosaminidase activity. Fast
Garnet histochemistry of murine fibroblasts exposed to (Figure 2A) conditioned medium
collected from BHK^{HexlacZ} cells compared to cells exposed to medium from normal parent
BHK-21 cells (Figure 2B). These results demonstrate that hHEXA & hHEXB, products of

5   the β-Hex transgene, are released into the extracellular medium and can be absorbed by
other cells via paracrine pathways resulting in induction of the cellular β-hexosaminidase.

2.   Example 2 Transfecting constructs

a)   Construction of the tricistronic β-Hex construct

[190]   A tricistronic construct encoding for both isoforms of human β-
10 hexosaminidase, hHexA & hHexB, as well as the β-galactosidase reporter gene (*lacZ*) was
also made. hHexB cDNA was isolated following *Xho I* digestion of pHexB43 (ATCC,
Manassas VA) and cloned into the *Xho* I site of pIRES (Clonetech Laboratories, Palo Alto
CA) downstream of the vector's cytomegalovirus (CMV) promoter sequence. The HexA
cDNA was isolated from pBHA-5 (ATCC, Manassas VA) by *Xho I* digestion and was
15 subsequently inserted into the *Xba I* site of pIRES(HexB) downstream of the vector's IRES
cassette by blunt ligation. A *IRES-lacZ* cassette was obtained from Dr. Howard J. Federoff,
University of Rochester School of Medicine and Dentistry, but can be produced using
standard recombinant techniques with known reagents and was inserted downstream to
HexA into the Sal I site of pHexB-IRES-HexA by blunt ligation. In this construct, the
20 cytomegalovirus promoter (CMV) drives transgene expression, and the translation of the
second and third open reading frames (ORF), *HexB* and *lacZ*, respectively, are facilitated by
an internal ribosomal entry sequence (IRES). The FIV(Hex) vector was constructed by
isolating the HexB-IRES-HexA (β-Hex) fragment of pHexlacZ with *NheI - NotI* digestion
is present and it was cloneed into the FIV backbone (Poeschla et al., 1998), derived after
25 excising the lacZ cassette from pFIV(lacZ) with *Bpu1102I*, leading to the successful
construction of pFIV(Hex) (See Figures 3 and 4). Restriction fragment analysis indicated
that pFIV(Hex) was constructed as designed. (Figure 5).

[191]   The viral derived IRES sequence can effectively drive the expression of
second genes in bicistronic constructs *in vitro* and *in vivo*, (Gurtu *et al.*, 1996; Geschwind *et*
30 al., 1996; Havenga *et al.* 1998). Nevertheless, IRES-mediated transcription in bicistronic
constructs has been shown to reduce the levels of expression of the second ORF by about
40-50%. Hence, since HexB is necessary in the synthesis of both HEXA (α/β) and HEXB

(α/α), it was cloned first in our tricistronic construct. Confirmation of the construct has been achieved by multiple restriction enzyme digestions as well as direct DNA sequencing.

b) Results

[192]　The FIV backbone vector was isolated from the FIV(lacZ) vector following *Sst* II & *Not* I digestion. The bicistronic transgene *HexB*-IRES-*HexA* was extracted from the pHex*lacZ* vector following *Nhe* I & *Not* I digestion, and was cloned into the FIV backbone by blunt ligation. FIV(Hex) digestion with the restriction enzymes *Xho I* and *Sal I* confirmed the cloning. (Figure 6) FIV(Hex) virus was prepared using established methods and was tested *in vitro* as follows. Cultured murine fibroblasts (CrfK cell line) were exposed to FIV(Hex) for 12 hours, followed fresh media change. After 48 hours, cellular DNA and RNA extracts were collected. The presence of viral DNA was assessed by PCR with primers sets specifically designed for HexB (Figure 6A). HexB expression was assessed by RT-PCR (Figure 6B). These results demonstrate the ability of FIV(Hex) to transduce mouse fibroblasts with β-Hex, resulting in transgene mRNA expression. (Figure 6).

[193]　The tricistronic vector pHEXlacZ was stably expressed in embryonic hamster kidney fibroblasts (BHK-21; ATCC) following standard transfection laboratory techniques using the LIPOFECTAMINE ® reagent (Gibco BRL) per manufacturer's instructions. Forty-eight hours post-transfection, the cells were treated with 800μg/mL G418 (Gibco BRL) for 10 days, and cell lines were selected, expanded and analyzed for expression of our tricistronic gene as follows. Analysis of the transfected cells showed that cell lines (Crfk, spleen, brain, liver, and kidney) stained positively for X-gal, indicating expression of and translation of the expressed product from the tricistronic vector. (Figure 6)

3.　Example 4 In vivo use of FIV HEX vectors

[194]　FIV(Hex) was constructed by inserting the bicistronic gene HexB-IRES-HexA in the place of the reporter gene lacZ in the FIV backbone vector using standard mmolecular biology techniques. FIV(Hex) was prepared in vitro by transient co-transfection of the transfer vector along with the packaging and envelop plasmids into 293H cells. The virus-rich supernatant was centrifuged and the viral pellet was reconstituted in normal saline, and was then titered in CrfK cells by the X-Hex histochemical method (10^7-10^8 infectious particles/ml). The viral solution was injected intraperitoneally to 2 days old HexB$^{-/-}$ knockout mouse pups, which were allowed to reach the critical age of 16 weeks,

when they displayed full signs of the lysosomal storage disease. For control, littermates were

injected with the FIV(lacZ) virus, which is identical to FIV(Hex), but instead of carrying

the HexB-IRES-HexA gene it carries the reporter gene lacZ. Locomotive performance was

evaluated by placing the mice on a wire mesh attached on a clear plexiglass cylinder, and

5   turning the wire mesh up-side-down. The lapse time until the mice fell off the wire mesh

was recorded on weekly basis until the mice were terminated. It is important to state that at

the critical time point of 16 weeks, the FIV(Hex) injected mice showed statistically better

locomotive performance compared to FIV(lacZ) injected mice (controls). Furthermore, the

FIV(Hex) mice had an extended life span for at least 2-3 additional weeks, at which point

10   they were also terminated because they were showing signs of the disease.

4. Example 3 HIV HEX vectors

[195] The HexB-IRES-HexA therapeutic gene was cloned into the Lenti6/V5D-

TOPO vector commercially available by Invitrogen (Carlsbad, CA), whereby the

cytomegalovirus promoter CMV drives gene expression [in a manner similar to FIV(Hex)].

15   A virus was constructed whereby the expression of HexB-IRES-HexA is driven by a

promoter, such as that show in SEQ ID NO:23, which consists of a beta-actin portion and a

CMV portion. This type of promoter has high expression in mammalian cells.

G. References

20   [196] Akli S, Guidotti J-E, Vigne E, Perricaudet M, Sandhoff K, Kahn A, Poenaru

L (1996) Restoration of hexosaminidase A activity in human Tay-Sachs fibriblasts via

adenoviral vector mediated gene transfer. Gene Therapy 3: 769-774

[197] Bowers WJ, Howard DF, Federoff HJ (2000). Discordance between

expression and genome transfer titering of HSV amplicon vectors: recommendation for

25   standardized enumeration. Mol Ther 1:294-9.

[198] Burns JC, Friedmann T, Driever W, Burrascano M, Yee J-K (1993)

Vescicular stomatitis virus G-glycoprotein pseudotyped retroviral vectors Concentration to

very high titer and efficient gene transfer into mammalian and nonmammalian cells. Proc

Natl Acad Scie USA 90: 8033-8037.

30   [199] Cannon PM, Anderson WF (2000) Retroviral vectors for gene therapy. *In*

Gene Therapy: therapeutic mechanisms and strategies. NS Templeton and DD Lasic,

Editors. Marcel Dekker Inc, New York, pp 1-16.

[200] Daly TM, Okuyama T, Vogler C, Haskins ME, Muzyczka N, Sands MS (1999a) Intracranial injection of recombinant adeno-associated virus improves cognitive function in a murine model of mucopolysaccharidosis type VII.Hum Gene Ther 10:85-94.

[201] Daly TM, Vogler C, Levy B, Haskins ME, Sands MS (1999b) Neonatal gene transfer leads to widespread correction of pathology in a murine model of lysosomal disease. Proc Natl Acad Sci USA 96: 2296-2300.

[202] Enlow DH, Hans MG (1996) Essentials of facial growth. W.B.Saunders Co, New York.

[203] Goldstein GW, Betz AL, Bowman PD, Dorovini-Zis K (1986) In vitro studies of the blood-brain barrier using isolated brain capillaries and cultured endothelial cells. Ann NY Acad Sci 481:202-13.

[204] Gorlin RJ, Cohen MM, Levin LS (1990). SYNDROMES OF THE HEAD AND NECK, 3rd Edition. Oxford university Press, New York.

[205] Guidotti JE, M. A., Haase G, Caillaud C, McDonell N, Kahn A, Poenaru L (1999) Adenoviral gene therapy of the Tay-Sachs disease in hexosaminidase A deficient knockout mice. Hum Mol Genet 8: 831-838.

[206] Kjaer I, Keeling JW, Hansen BF (1999) The prenatal human cranium-Normal and pathologic development. Munksgaard, Copenhagen, pp 20-51.

[207] Kordower JH, Bloch J, Ma SY, Chu Y, Palfi S, Roitberg BZ, Emborg M, Hantraye P, Deglon N, Aebischer P (1999) Lentiviral gene transfer to the nonhuman primate brain. Exp Neurol 160: 1-16.

[208] Kyrkanides S, Kjaer I, Fisher-Hansen B (1993) The basilar part of the occipital bone in normal and pathological fetuses. J Cranio Genet Develop Biol 13: 184-192.

[209] Kyrkanides S, Bellohusen R, Subtelny JD (1995) Skeletal asymmetries of the nasomaxillary complex in non-cleft and post-surgical unilateral cleft lip and palate individuals. Cleft Pal Craniofac J 32: 428-32.

[210] Kyrkanides S, Bellohusen R, Subtelny JD (1996) Asymmetries of the upper lip and nose in non-cleft and post-surgical unilateral cleft lip and palate individuals. Cleft Palate-Craniofacial Journal, 33:306-10.

[211] Kyrkanides S, Olschowka JA, Williams JP, Hansen JT, O'Banion MK (1999). TNFα and IL-1β mediate ICAM-1 induction via microglia-astrocyte interaction in CNS radiation injury. J Neuroimmunol 95:95-106.

[212] Kyrkanides S, Klambani M, Subtelny JD (2000). Cranial base and facial skeleton asymmetries in unilateral cleft lip & palate individuals. Cleft Palate-Craniofacial Journal 37: 556-561.

[213] Kyrkanides S, Olschowka JA, Whitley P, O'Banion MK (2001). Enhanced glial activation and expression of specific CNS inflammation-related molecules in aged versus young rats following cortical stab injury. *In Press* , J Neuroimmunol.

[214] Lipschutz GS, Gruber CA, Cao Y, Hardy J, Contag CH, Gaensler KML (2001). *In utero* delivery of adeno-associated viral vectors: Intraperitoneal gene transfer produces long term expression. Mol Ther 3: 284-92.

[215] McCormack JE, Edwards W, Sensintaffer J, Lillegren L, Kozloski M, Brumm D, Karavodin L, Jolly DJ, Greengard J (2001) Mol Ther 3: 516-525.

[216] Phaneuf D, Wakamatsu N, Huang J-Q, Borowski A, Peterson AC, Fortunato SR, Ritter G, Igdoura SA, Morales CR, Benoit G, Akerman BR, Leclerc D, Hanai N, Marth JD, Trasler JM, Gravel RA (1996) Dramatically different phenotypes in mouse models of human Tay-Sachs and Sandhoff diseases. Hum Mol Genet 5: 1-14.

[217] Poeschla EM, Wong-Stall F, Looney DL (1998) Efficient transduction of nondividing human cells by feline immunodeficiency virus lentiviral vectors. Nature Medicine 4: 354-357.

[218] Proia RL, d'Azzo A, Neufeld EF (1984). Association of a- and b- subunits during the biosynthesis of b-hexosaminidase in cultured human fibroblasts. J Biol Chem 259: 3350-3354.

[219] Risau W, Hallmann R, Albrecht U (1986) Differentiatiom-dependent expression of proteins in brain endothelium during development of the blood-brain barrier. Devel Biol 117: 537-545.

[220] Risau W, Wolburg H (1990) Development of blood-brain barrier. *TINS* 13:174-178.

[221] Rosenberg A (1999) Bones, joints and soft tissue tumors. *In* Robbins Pathologic Basis of Disease, 6th edition; Cotran, Kumar & Collins (Ed), W.B.Saunders Co, New York.

[222] Sakurada H, Itoh K, Kuroki Y, Kase R, Shimmoto M, Utsumi K, Ozawa H, Tai T, Hara A, Uyama E (1997) Immunocytochemical detection of accumulated substrates

in cultured fibroblasts from patients with the infantile and adult forms of Sandhoff disease. Clin Chim Acta 265: 263-266.

[223] Sango K, McDonald MP, Crawley JN, Mack ML, Tifft CJ, Skop E, Starr CM, Hoffmann A, Sandhoff K, Suzuki K, Proia RL (1996) Mice lacking both subunits of lysosomal β-hexosaminidase display gangliosidosis and mucopolysaccharidosis. Nature Genet 14: 348-352.

[224] Sango K, Yamanaka S, Hoffman A, Okuda Y, Grinberg A, Westphal H, McDonald MP, Crawley JN, Sandhoff K, Suzuki K, Proia RL (1995) Mouse models of Tay-Sachs and Sandhoff diseases differ in neurologic phenotype and ganglioside metabolism. Nature Genet 11: 170-176.

[225] Schroeder WH, Mortari F, Shiokawa S, Kirkham PM, Elgavish RA, Bertrand FE III (1995) Developmental regulation of the human antibody repertoire. Ann NY Acad Scie 764: 242-260.

[226] Suzuki K, Sango K, Proia RL, Langman C (1997) Mice deficient in all forms of lysosomal β-hexosaminidase show mucopolysaccharosis-like pathology. J Neuropath Exp Neurol 56: 693-703.

[227] Tarantal AF, O'Rourke JP, Case SS, Newbound GC, Li J, Lee CI, Baskin CR, Kohn DB, Bunnell BA (2001) Rhesus monkey model for fetal gene transfer: studies with retroviral-based vector systems. Mol Ther 3: 128-138.

[228] Wolff JA, Harding CO (2000) Principles of gene therapy for inborn errors of metabolism. *In* Gene Therapy: therapeutic mechanisms and strategies. NS Templeton and DD Lasic, Editors. Marcel Dekker Inc, New York, pp 507-533.

H. Sequences

1. SEQ ID NO:1 Homo sapiens hexosaminidase A (alpha polypeptide) (HEXA), Genbank Accession No. XM_037778

2. SEQ ID NO:2 Homo sapiens hexosaminidase A (alpha polypeptide) (HEXA), Genbank Accession No. XM_037778

3. SEQ ID NO:3 Homo sapiens hexosaminidase B (beta polypeptide) (HEXB), protein Genbank Accession No XM_032554

4. SEQ ID NO:4 Homo sapiens hexosaminidase B (beta polypeptide) (HEXB), mRNA Genbank Accession No XM_032554

5. SEQ ID NO:5 IRES sequence United States Patent No. 4,937,190 herein incorporated by reference covers entire Vector, and is cited at least for material relating to the pIRES vector)

6. SEQ ID NO:6 Mus musculus hexosaminidase A (Hexa), protein Genbank Accession No, NM_010421

7. SEQ ID NO:7 Mus musculus hexosaminidase A (Hexa), mRNA Genbank Accession No, NM_010421

8. SEQ ID NO:8 FIV(LacZ) construct 12750 bp

9. SEQ ID NO:9: HEX-α polypeptide Genbank accession number NM_000520 (Proia) beta-hexosaminidase A alpha-subunit to human chromosomal region 15q23----q24

10. SEQ ID NO:10 HexA gene Genbank accession number NM_000520 (Proia)

11. SEQ ID NO:11 HexB degenerate cDNA G to A change at position 6

12. SEQ ID NO:12: HEX-β polypeptide conservative substitution of Val21 to I21

13. SEQ ID NO:13 HEX-α polypeptide Genbank accession number M16411 (Tissue sample from ATCC)

14. SEQ ID NO:14 HexA gene Genbank accession number M16411

15. SEQ ID NO:15: HEX-β polypeptide Genbank accession number NM_000521 (Proia) beta-hexosaminidase A alpha-subunit to human chromosomal region chromosome 5 map="5q13"

16. SEQ ID NO:16 HexB gene Genbank accession number NM_000521 Proia

17. SEQ ID NO:17 Mus musculus hexosaminidase B (Hexb), protein. Genbank Accession No. NM_010422

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18. SEQ ID NO:18 Mus musculus hexosaminidase B (Hexb), mRNA. Genbank Accession No. NM_010422

19. SEQ ID NO:19 Bactin Hex sequence

20. SEQ ID NO:20 HIV Hex vector sequence

5   21. SEQ ID NO:21 E02199 DNA encoding chicken beta actin gene promoter.

22. SEQ ID NO:22 Chicken Beta Actin promoter

23. SEQ ID NO:23 CMV-Beta actin promoter

24. SEQ ID NO:24 Fusion promoter-CMV portion

25. SEQ ID NO:25 Fusion promoter – beta actin portion

10   26. SEQ ID NO:26 Chicken beta actin promoter

27. SEQ ID NO:27 Accession # BD136067. promoter element for sustained gene expression from CMV promoter.

28. SEQ IDNO:28 BD136066 Accession # promoter element for sustained gene expression from CMV promoter.

15   29. SQ ID NO:29 BD136065 Accession # promoter element for sustained gene expression from CMV promoter.

30. SEQ ID NO:30 BD136064 Accession # promoter element for sustained gene expression from CMV promoter

31. SED ID NO:31 L77202 Accession # Murine Cytomegalovirus early (E1) gene,
20   promoter region.

32. SEQ ID NO:32 X03922 Accession # Human cytomegalovirus (HCMV) IE1 gene promoter region.

33. SEQ ID NO:33 E06566 Accession # Promoter gene of human beta-actin gene.

34. SEQ ID NO:34 E02198 Accession # Dna encoding 3'end region of beta-actin
25   gene promoter

35. SEQ ID NO:35 E02197 Accession # DNA encoding 3'end region of beta-actin gene promoter.

36. SEQ ID NO:36 E02196 Accession # DNA encoding 3'end region of beta-actin gene promoter.

30   37. SEQID NO:37 E02195 Accession # DNA encoding 3'end region of beta-actin gene promoter.

38. EQ ID NO:38 E02194 Accession # DNA encoding chicken beta-actin gene promoter.

39. SEQ ID NO:39 E01452 Accession # Genomic DNA of promoter of human beta-actin.

5   **40. SEQ ID NO E03011 Accession # DNA encoding hybrid promoter that is composed of chicken beta-actin gene promoter and rabbit beta-globin gene promoter.**

41. SEQ ID NO:41 BD015377 Accession # Baculovirus containing minimum CMV promoter.

10   **42. Other cytomegalovirus promoter regions**

[229]   Other human cytomegalovirus promoter regions can be found in accession numbers M64940, Human cytomegalovirus IE-1 promoter region, M64944 Human cytomegalovirus IE-1 promoter region, M64943 Human cytomegalovirus IE-1 promoter region, M64942 Human cytomegalovirus IE-1 promoter region, M64941 Human

15   cytomegalovirus IE-1 promoter region (All of which are herein incorporated by reference at least for their sequence and information)

VI.   CLAIMS

What is claimed is:

1. A composition comprising a nucleic acid wherein the nucleic acid comprises a sequence encoding a HEX-α and a sequence encoding a HEX-β.

2. The composition of claim 1, wherein the sequence encoding the HEX-β is orientated 5' to the sequence encoding HEX-α.

3. The composition of claim 1, further comprising a promoter.

4. The composition of claim 1, further comprising an integrated ribosomal entry site (IRES).

5. The composition of claim 4, wherein the sequence encoding the HEX-β is orientated 5' to the IRES sequence and the IRES sequence is located 5' to the sequence encoding HEX-α.

6. The composition of claim 4, further comprising a promoter.

7. The composition of claim 6, wherein the promoter is located 5' to the sequence encoding the HEX-β and the sequence encoding the HEX-β is orientated 5' to the IRES sequence and the IRES sequence is located 5' to the sequence encoding HEX-α.

8. The composition of claim 6, wherein the parts are oriented 5'-promoter- HEX-β encoding sequence-IRES- HEX-α encoding sequence-3'.

9. The composition of claim 6, wherein the parts are oriented 5'-promoter- HEX-α encoding sequence -IRES- HEX-β encoding sequence -3'.

10. The composition of claim 6, wherein the nucleic acid comprises a second IRES sequence.

11. The composition of claim 10, wherein the second IRES sequence is located 3' to the other parts.

12. The composition of claim 6, wherein the HEX-β has at least 70%, 75%, 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO:3 and the HEX-α has at least 70%, 75%, 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO:1.

13. The composition of claim 12, wherein any change from SEQ ID NO:3 or SEQ

ID NO:1 is a conservative change.

14. The composition of claim 13 wherein the HEX-β has the sequence set forth in SEQ ID NO:3 and the HEX-α has the sequence set forth in SEQ ID NO:1.

15. The composition of claim 6, wherein the sequence encoding HEX-β hybridizes to SEQ ID NO:2 under stringent conditions and wherein the HEX-α element hybridizes to SEQ ID NO:4 under stringent conditions.

16. The composition of claim 12, wherein the IRES sequence comprises a sequence having at least 70%, 75%, 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO:5.

17. The composition of claim 16, wherein the promoter sequence comprises a constitutive promoter.

18. The composition of claim 17, wherein the promoter sequence comprises a CMV promoter.

19. The composition of claim 18, wherein the CMV promoter comprises the sequence set forth in SEQID NO:32.

20. The composition of claim 16, wherein the promoter sequence comprises a beta actin promoter.

21. The composition of claim 20, wherein the beta actin promoter sequence comprises an avian beta actin promoter sequence.

22. The compositin of claim 21, wherein the beta actin promoter sequence comprises a mammalian beta actin promoter sequence.

23. The composition of claim 21, wherein the beta actin promoter comprises the sequence set forth in SEQ ID NO:26.

24. The composition of claim 16, wherein the promoter sequence comprises an inducible promoter.

25. The composition of claim 18, wherein the promoter sequence further comprises a beta actin promoter.

26. The composition of claim 6, wherein the composition produces a functional HEXB product.

27. The composition of claim 6, wherein the composition produces a functional HEXA product.

28. The composition of claim 6, wherein the composition produces a functional HEXS product.

29. The composition of claim 26, wherein the composition is capable of cross correcting.

30. The composition of claim 26, wherein the function is the catabolism of GM2 gangliosides in mammalian cells. Same for HEXB, the homodimer of HexB/HexB.

31. The composition of claim 6, wherein the nucleic acid further comprises a reporter gene.

32. The composition of claim 31, wherein the reporter gene is a lacZ gene.

33. The composition of claim 31, wherein the reporter gene is flanked by recombinase sites.

34. The composition of claim 33, wherein the recombinase sites are for the cre recombinase.

35. The composition of claim 6, wherein the nucleic acid further comprises a transcription termination site.

36. The composition of claim 35, wherein the transcription termination site is oriented 5' to the promoter sequence.

37. The composition of claim 36, wherein the transcription termination site is flanked by recombinase sites.

38. The composition of claim 37, wherein the recombinase sites are for the cre recombinase.

39. The composition of claim 6, further comprising a vector.

40. The composition of claim 39, wherein the vector comprises a lentiviral vector.

41. The composition of claim 40, wherein the lentiviral vector comprises a feline immunodeficiency virus.

42. The composition of clam 40, wherein the lentiviral vector comprises a human immunodeficiency virus.

43. The composition of claim 39, wherein the vector can be stably integrated for at least three months.

44. A composition comprising a cell wherein the cell comprises the nucleic acid of claim 6.

45. A composition comprising a cell wherein the cell comprises the vector of claim 39.

46. The composition of claim 47, wherein the cell comprises a neuron, glia cell, fibroblast, chondrocyte, osteocyte, endothelial cell, or hepatocyte.

47. The composition of claims 6, wherein the composition is in pharmaceutically acceptable form.

48. The composition of claims 6, wherein the composition is in an effective dosage.

49. The composition of claim 48, wherein the effective dosage is determined as a dosage that reduces the effects of Tay Sachs or Sandoff's disease.

50. A composition comprising an animal wherein the animal comprises the vector of claim 39.

51. A composition comprising an animal wherein the animal comprises the nucleic acid of claim 6.

52. A composition comprising an animal wherein the animal comprises the cell of claim 45.

53. The composition of claim 50, wherein the animal is mammal.

54. The composition of claim 53, wherein the mammal is a murine, ungulate, or non-human primate.

55. The method of claim 54, wherein the mammal is a mouse, rat, rabbit, cow, sheep, or pig.

56. The composition of claim 54, wherein the mammal is mouse.

57. The composition of claim 56, wherein the mouse comprises a HexB knockout.

58. Thecomposition of claim 56, wherein the mouse comprises a HexA knockout.

59. The composition of claim 58, wherein the mouse further comprises a HexB

knockout.

60. The composition of claim 54, wherein the mammal is a non-human primate.

61. A method of providing HEXA in a cell comprising transfecting the cell with the nucleic acids of claims 6.

62. A method of providing HEXB in a cell comprising transfecting the cell with the nucleic acids of claims 6.

63. A method of providing HEX-α and HEX-β in a cell comprising transfecting the cell with the nucleic acid of claims 6.

64. The method of claim 63, wherein the step of transfecting occurs in vitro.

65. The method of claim 63, wherein the step of transfecting occurs in vivo.

66. A method of providing HEXS in a cell comprising transfecting the cell with the nucleic acids of claims 6.

67. A method of making a transgenic organism comprising administering the nucleic acid of claims 6.

68. A method of making a transgenic organism comprising administering the vector of claim 39.

69. A method of making a transgenic organism comprising administering the cell of claims 45.

70. A method of making a transgenic organism comprising transfecting a lentiviral vector to the organism at during a perinatal stage of the organism's development.

71. A method of treating a subject having Tay Sachs disease and/or Sandoff disease comprising administering the composition of claim 47.

72. A method of making a composition, the composition comprising a nucleic acid molecule, wherein the nucleic acid molecule is produced by the process comprising linking in an operative way a promoter element, an element comprising sequence encoding HEX-β, a IRES element, and an element encoding HEX-α.

73. The method of claim 72 wherein the HEX-β element comprises a sequence having at least 80% SEQ ID NO:1 and the HEX-α element comprises a sequence having at least 80% to SEQ ID NO:3.

74. The method of claim 73, wherein any change in SEQ ID NO:1 or SEQ ID NO:3 is a conservative change.

75. The method of claim 72, wherein the sequence encoding HEX-β hybridizes to SEQ ID NO:2 under stringent conditions and wherein the sequence encoding the HEX-α hybridizes to SEQ ID NO:4 under stringent conditions.

76. A method of producing a composition, the composition comprising a cell, the method comprising administering the nucleic acid of claim 6 to the cell.

77. A method of producing a composition, the composition comprising a peptide, the method comprising expressing the nucleic acid of claim 6.

78. The method of claim 77, further comprising isolating the peptide.

79. A method of producing a composition, the composition comprising an animal, the method comprising administering the nucleic acid of claim 6 to the animal.

80. The method of claim 79, wherein the animal is a mammal.

81. Wherein the mammal is a murine, ungulate, or non-human primate.

82. The method of claim 81, wherein the mammal is a mouse, rat, rabbit, cow, sheep, or pig.

83. A nucleic acid comprising a sequence encoding HEX-β wherein the HEX-β has the sequence set forth in SEQ ID NO:3, a sequence encoding HEX-α, wherein the HEX-α has the sequence set forth in SEQ ID NO:1, a promoter, and an IRES sequence, wherein the promoter is located 5' to the sequence encoding the HEX-β and the sequence encoding the HEX-β is orientated 5' to the IRES sequence and the IRES sequence is located 5' to the sequence encoding HEX-α.

84. A composition comprising a nucleic acid wherein the nucleic acid comprises a sequence encoding a first HEX-β and a sequence encoding a second HEX-β.

85. A composition comprising a nucleic acid wherein the nucleic acid comprises a sequence encoding a first HEX-α and a sequence encoding a second HEX-α.

86. A composition comprising four parts: 1) a promoter, 2) a sequence encoding a HEX-α, 3) a sequence encoding a HEX-β, and 4) an integrated ribosomal entry site (IRES).

FIG.1A

☐ HEX-A
▨ HEX-A & HEX-B
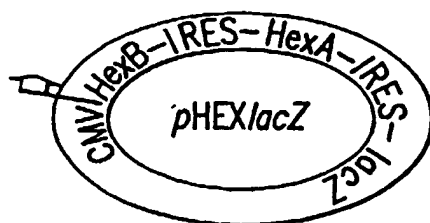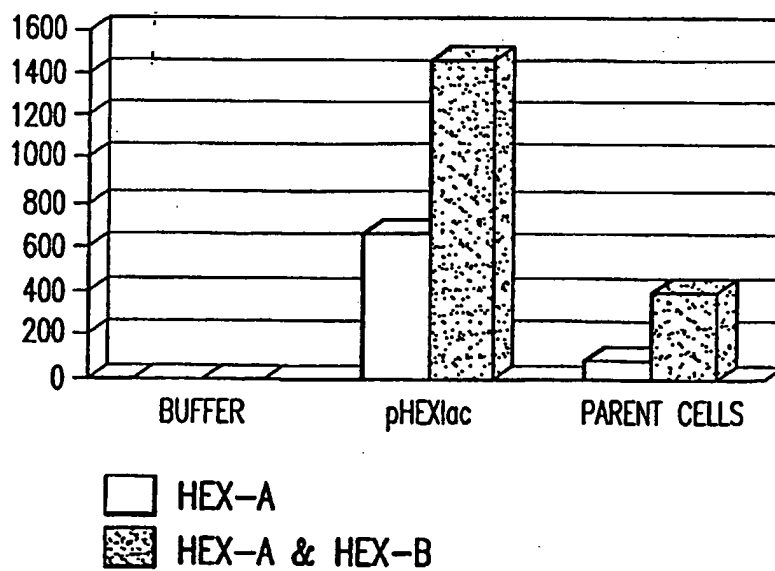
FIG.1F

FIG.1B
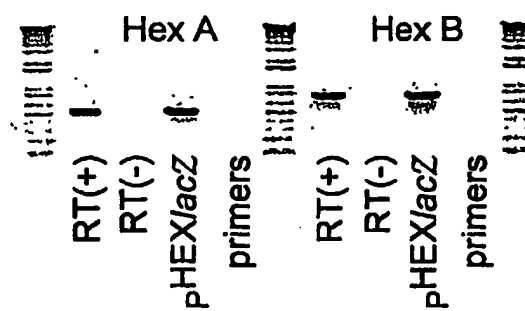
FIG.1C
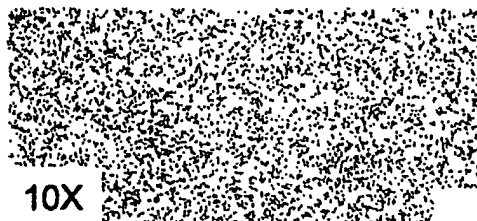
FIG.1D1

FIG.1D2
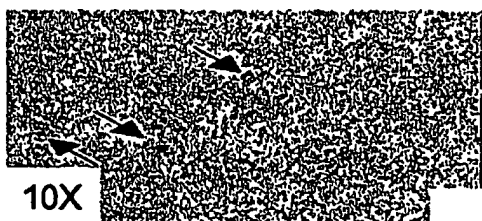
FIG.1E1

FIG.1E2

FIG.1G1   FIG.1G2
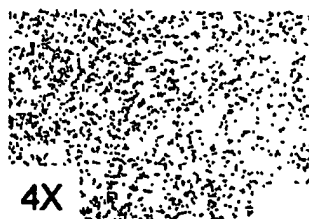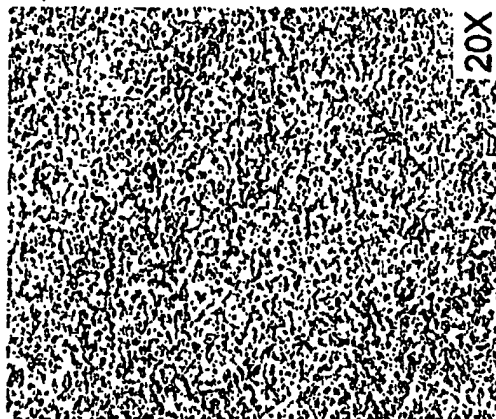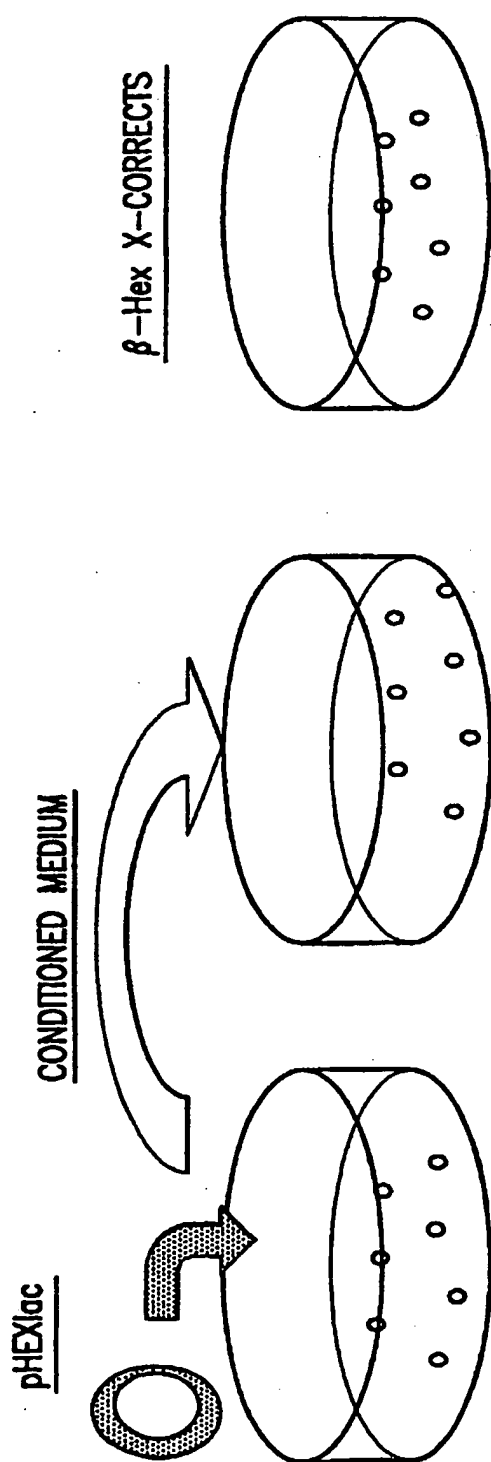
FIG.2A

FIG.2B
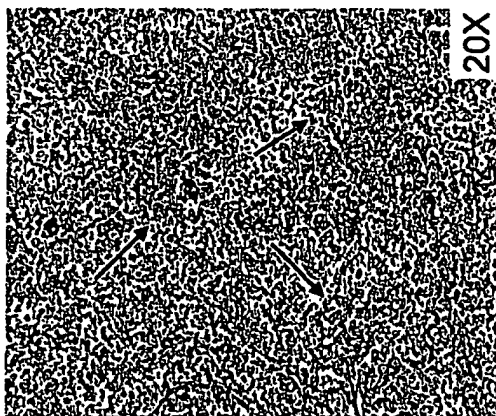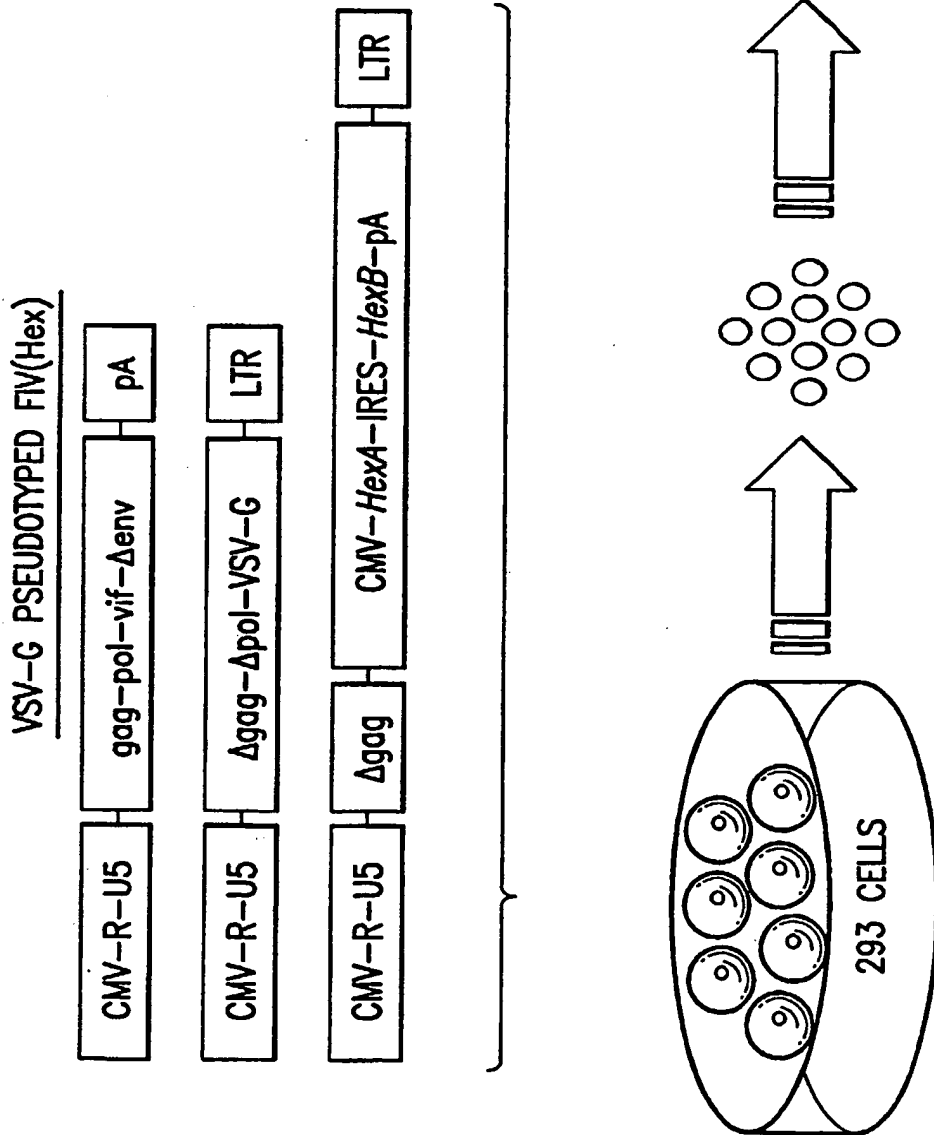
FIG.3
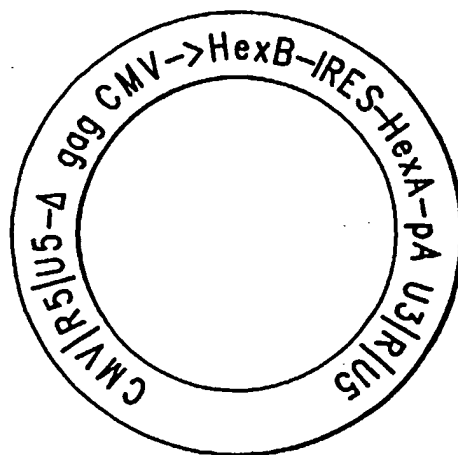
FIV (Hex)

FIG.4

FIV (Hex) 6.2 maxi prep   Total 1mg
1kb⁺ uncut Sca I Not I Sal I Xho I 1kb⁺   (1µg/µl)
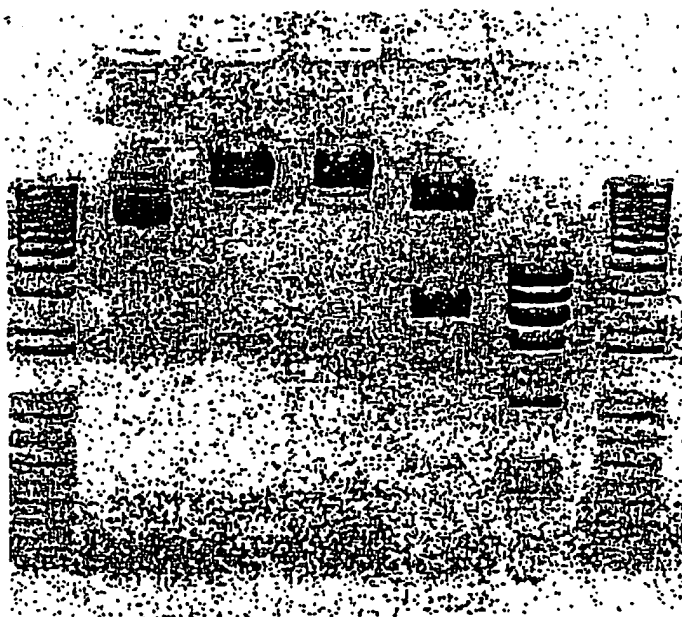
FIG.5
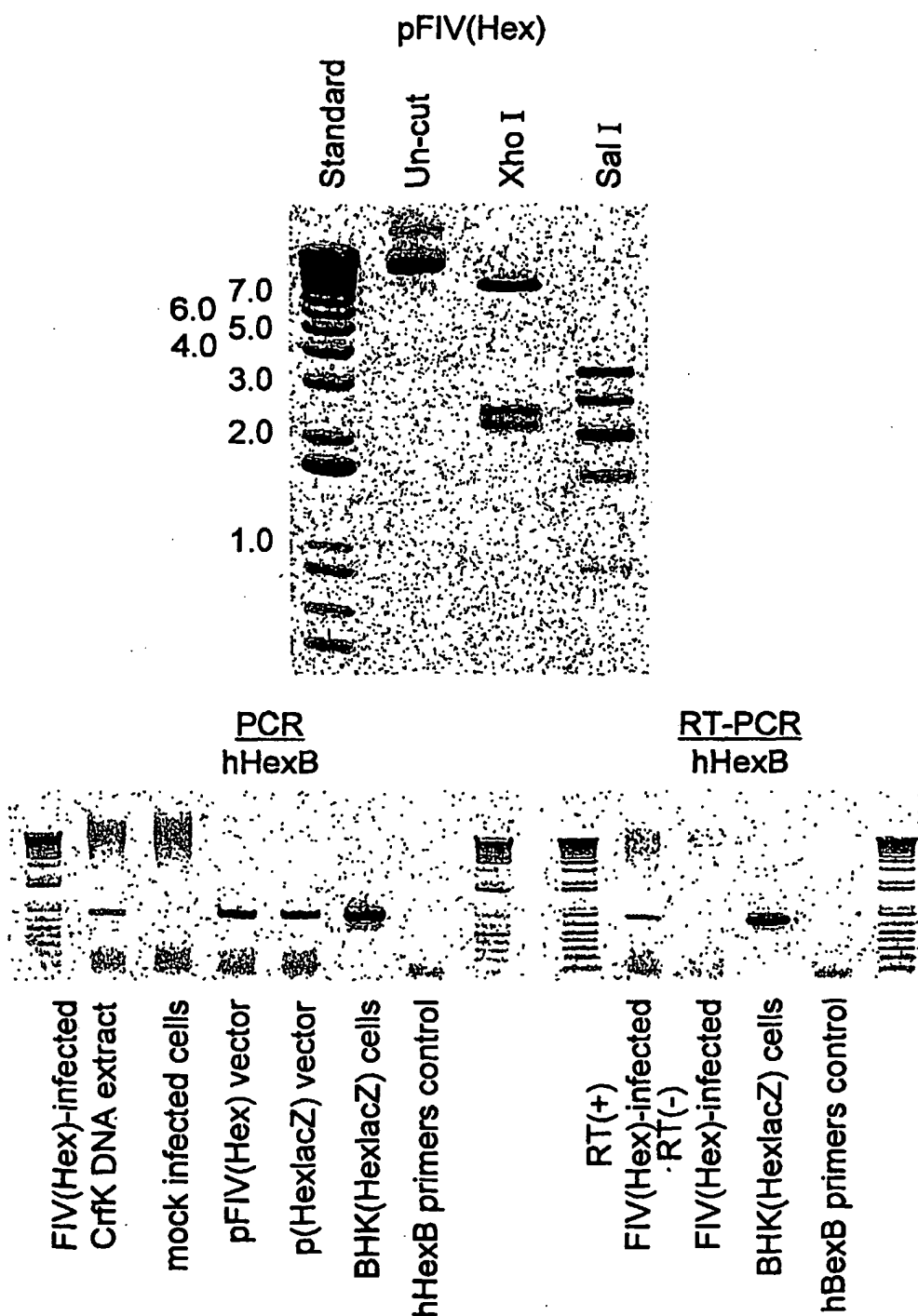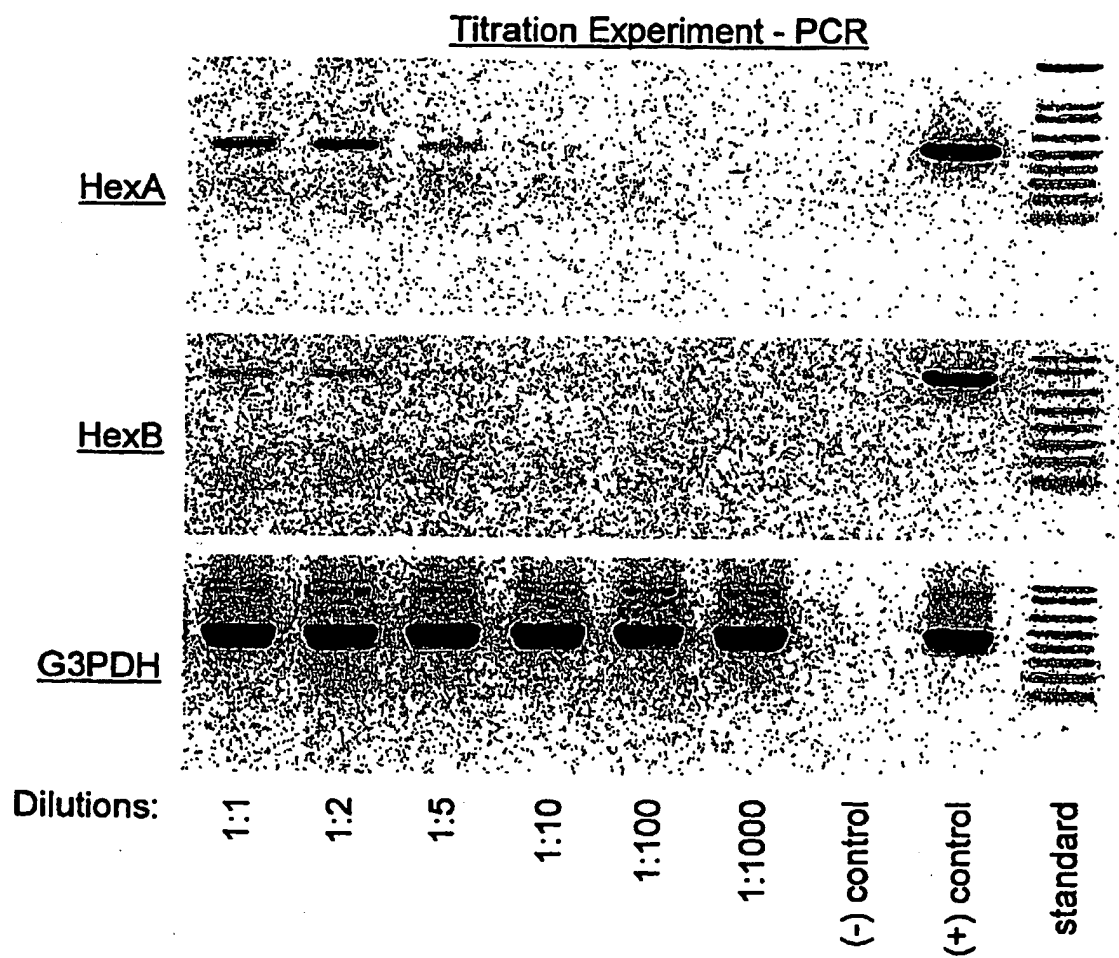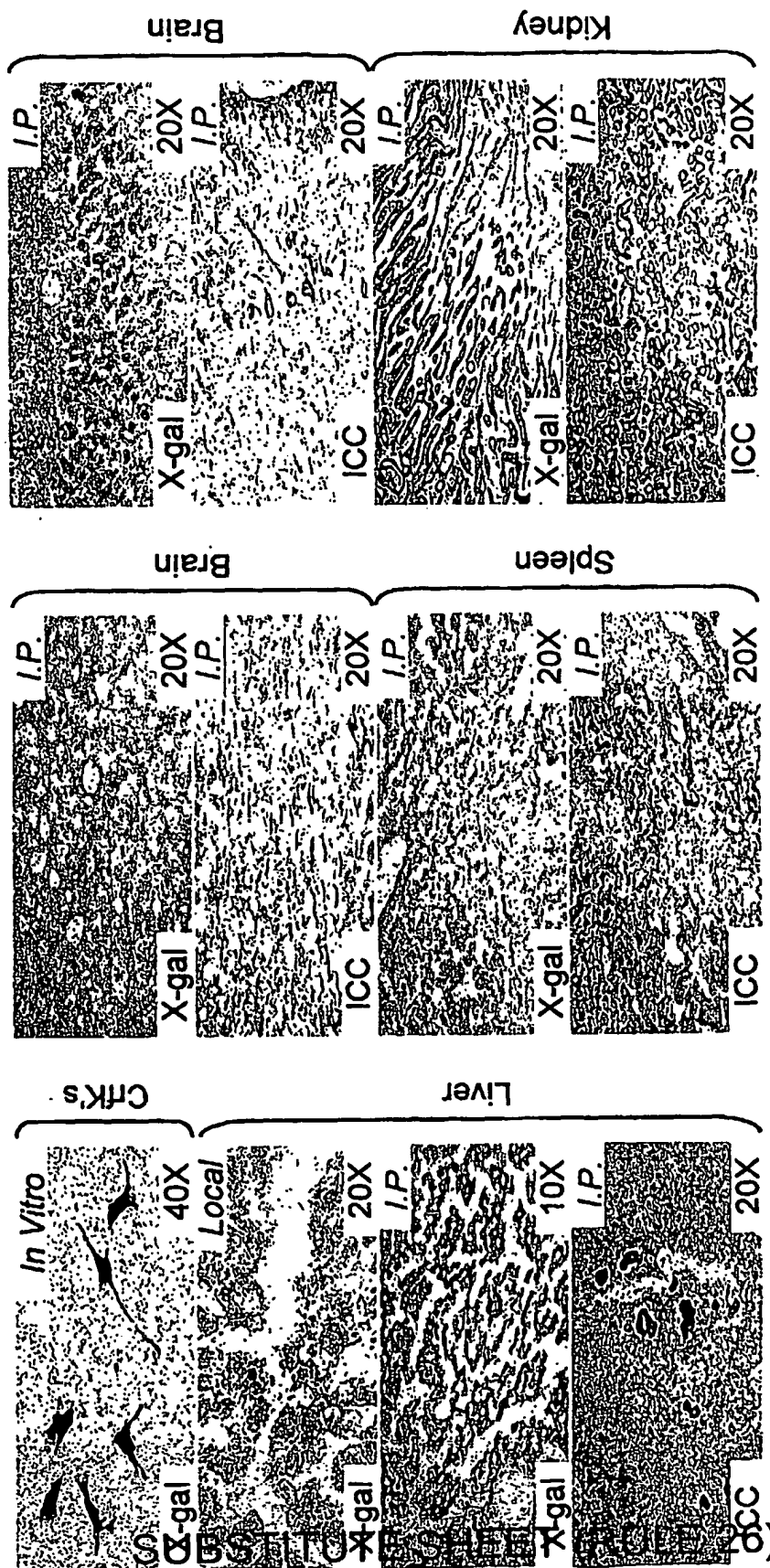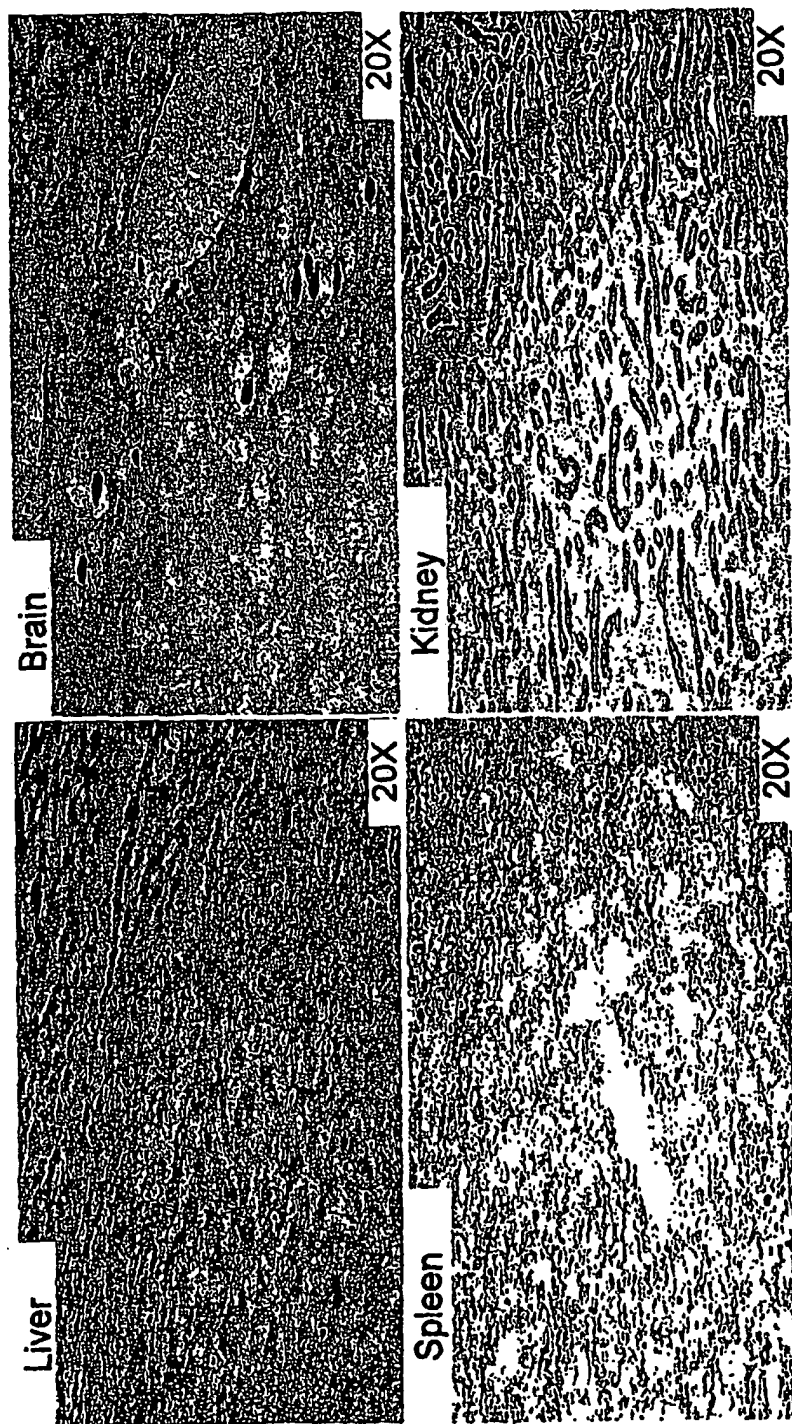
FIG.6

FIG.7

FIG.8

FIG.9
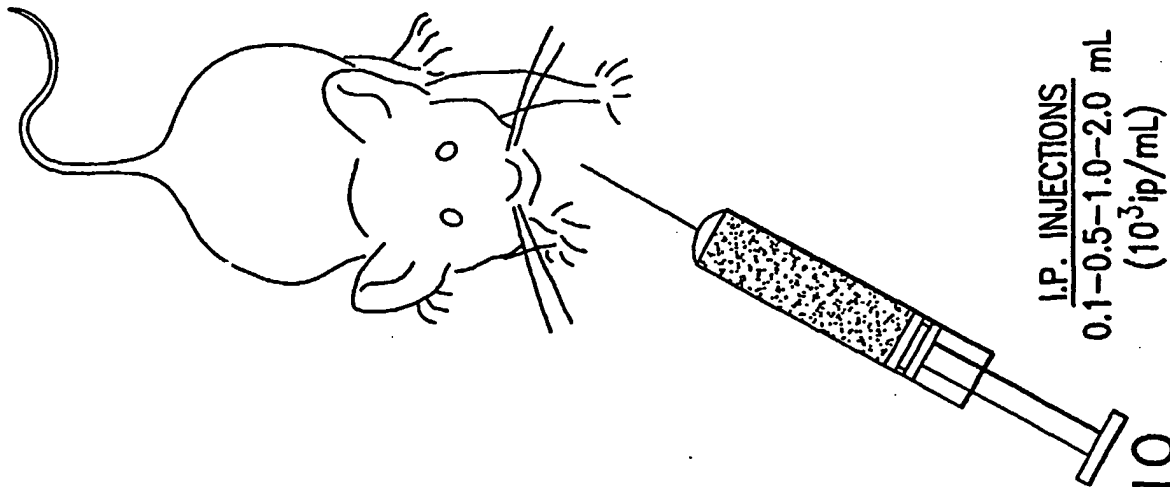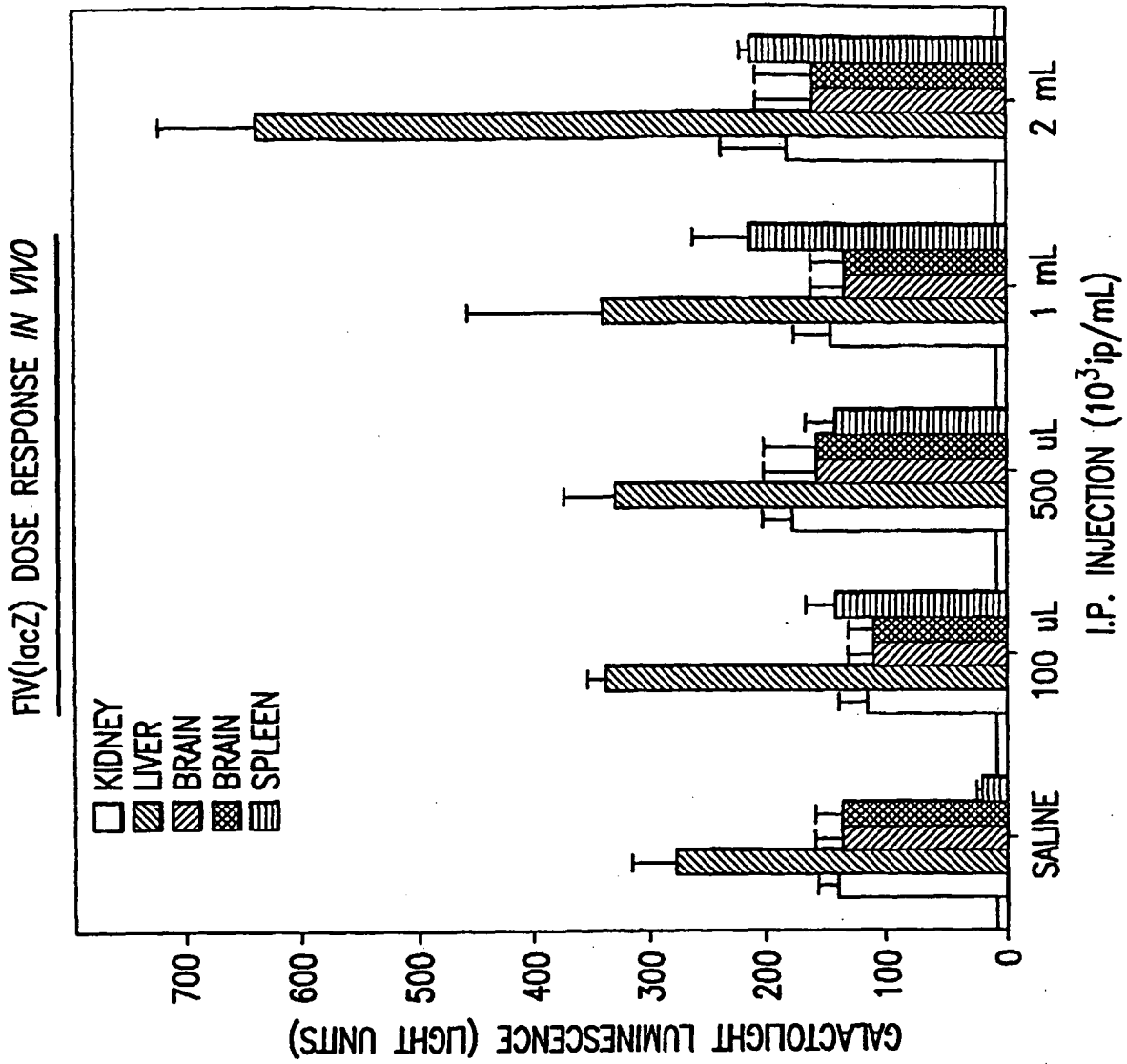
FIG. 10
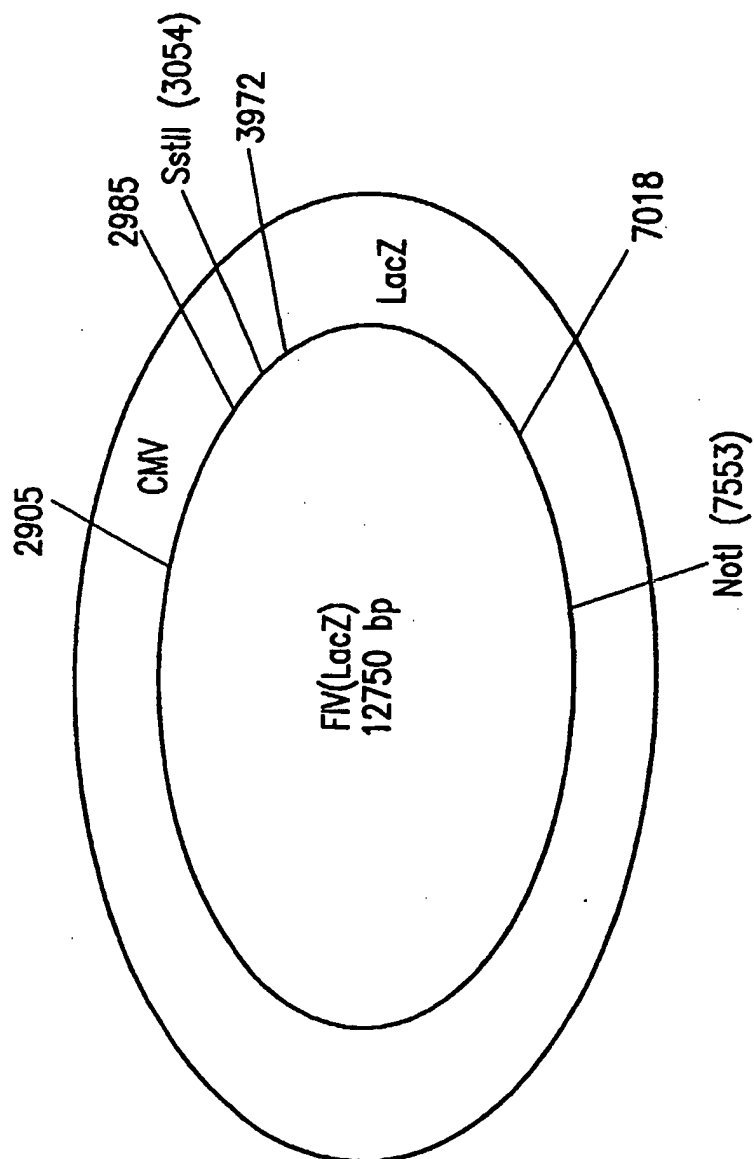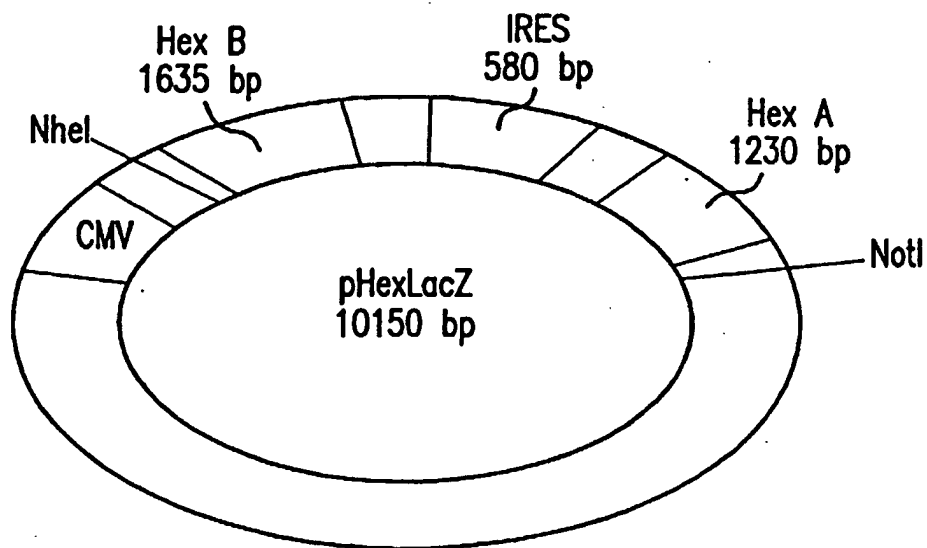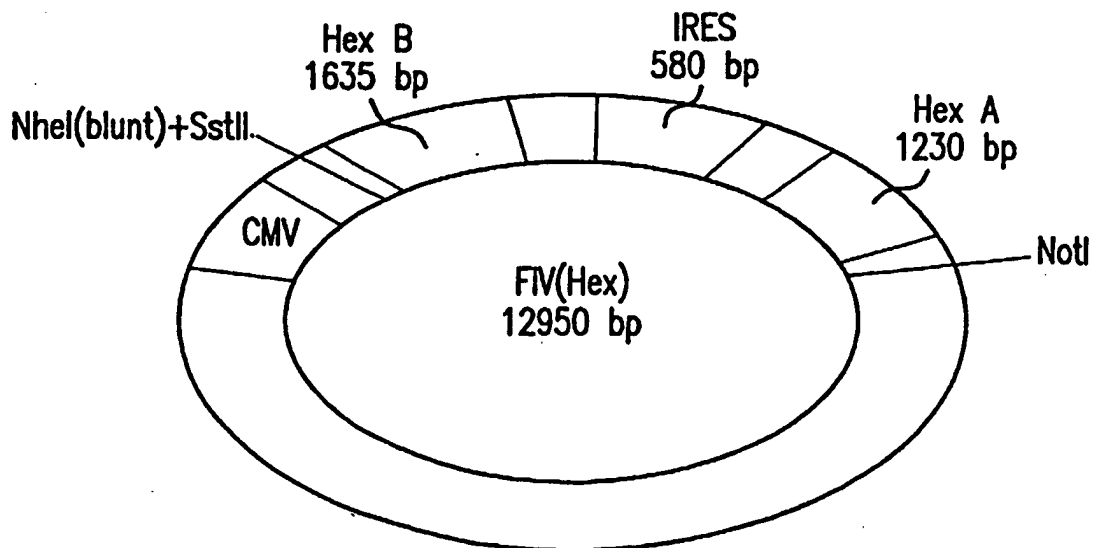
FIG.11

FIG.11A

FIG.11B
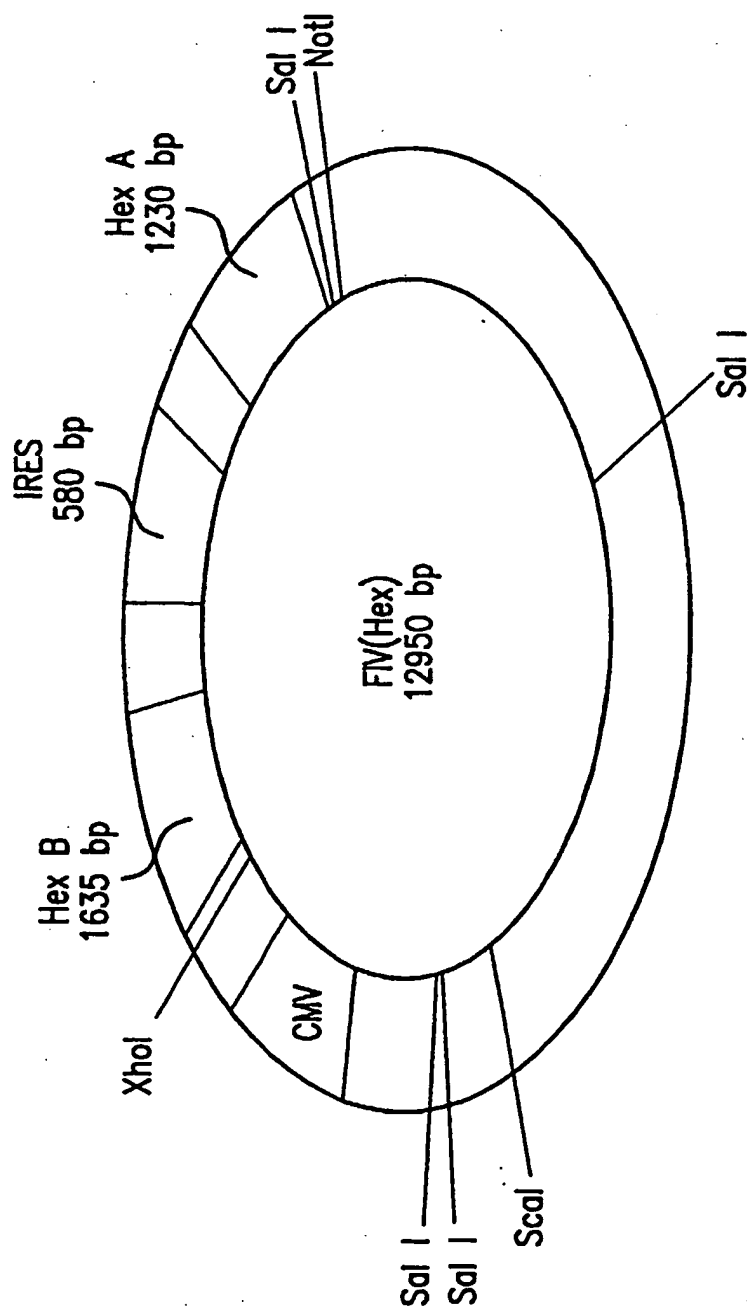
FIG.12
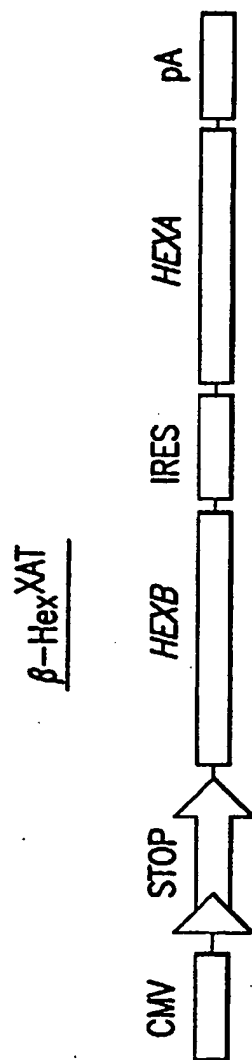
FIG.13
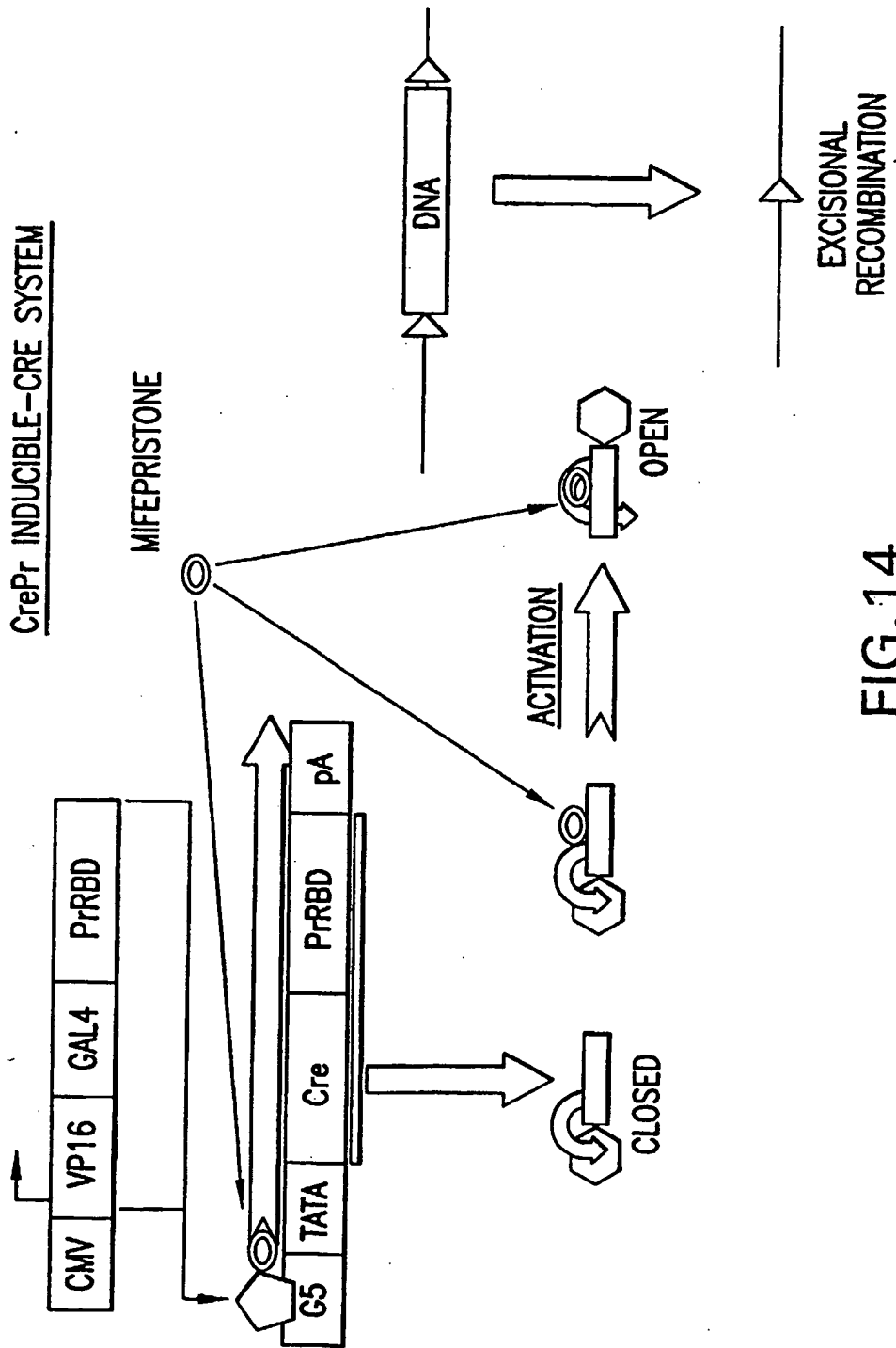
FIG.14
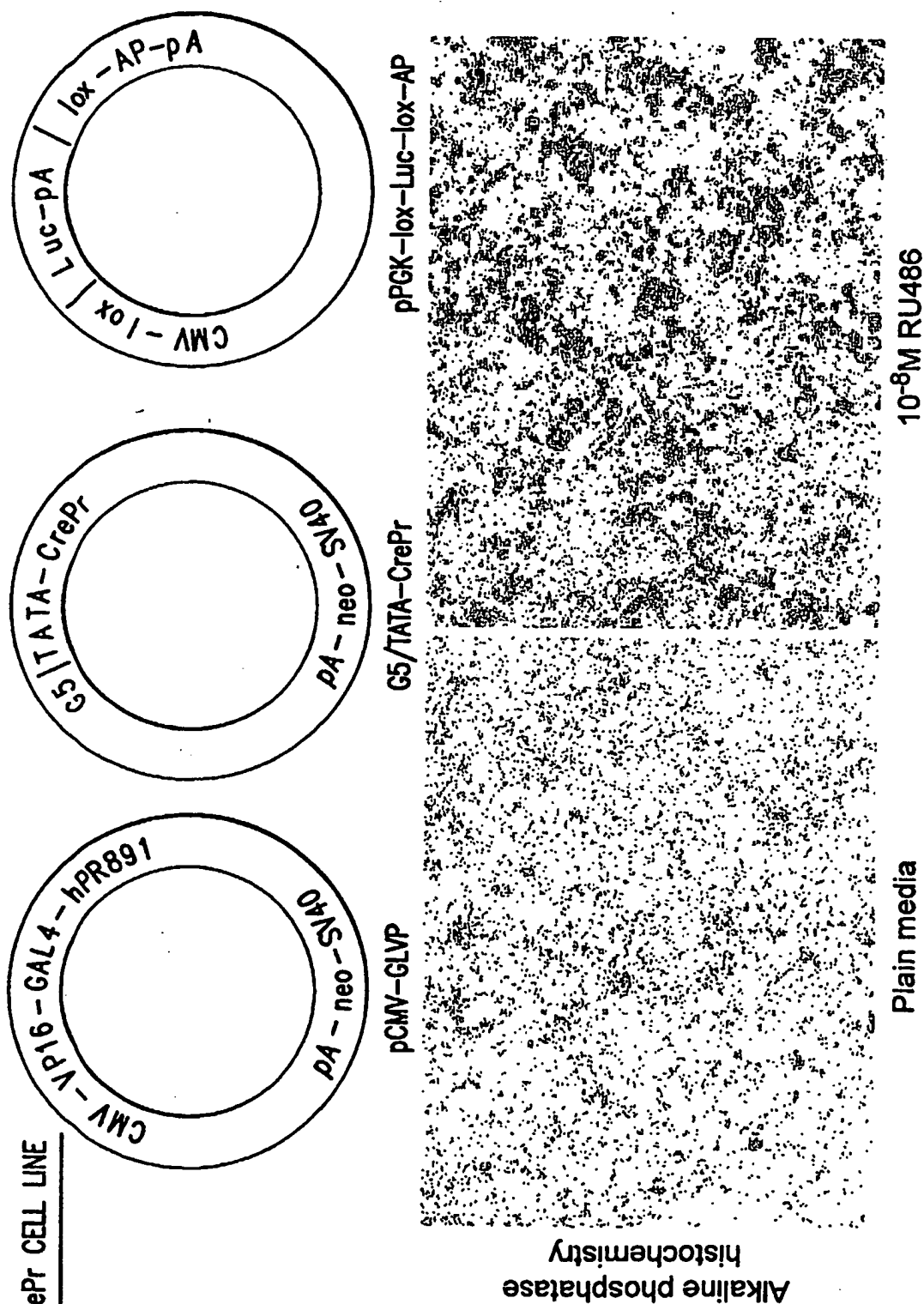
FIG.15

Hex^XAT: Excisionally activated β-hexosaminidase gene
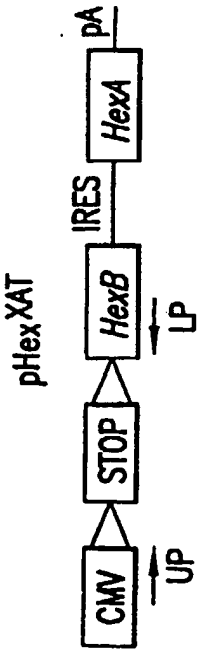
FIG.16A
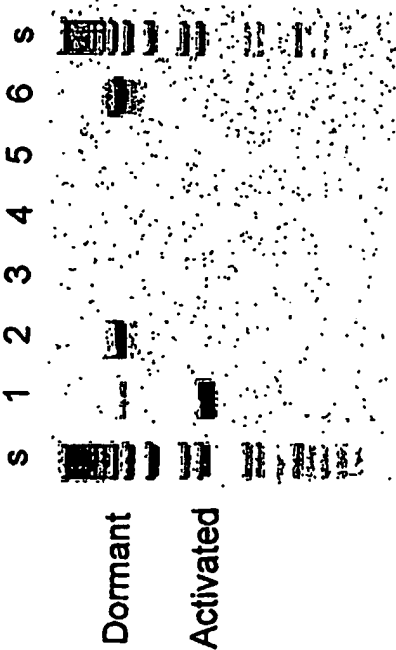
FIG.16B
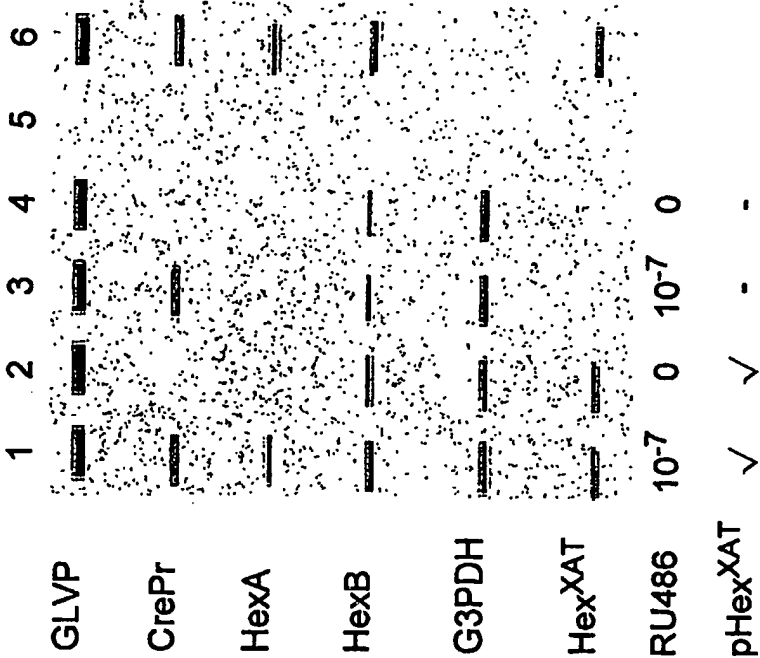
FIG.16C

HexXAT: EXCISIONALLY ACTIVATED β-HEXOSAMINIDASE GENE
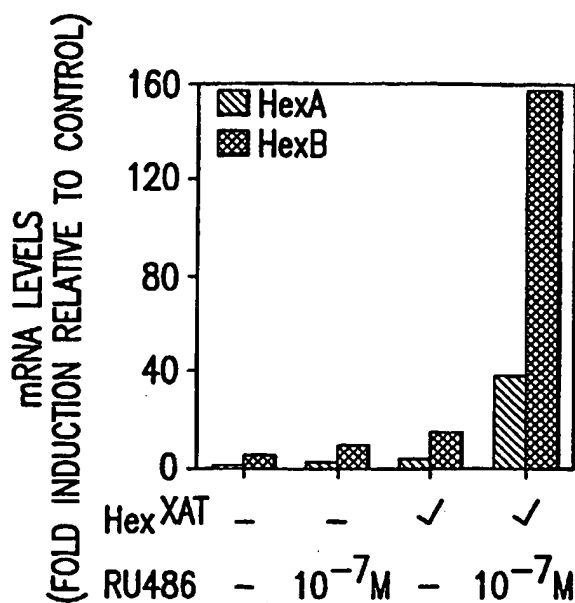
FIG.17A

HexXAT: EXCISIONALLY ACTIVATED β-HEXOSAMINIDASE GENE
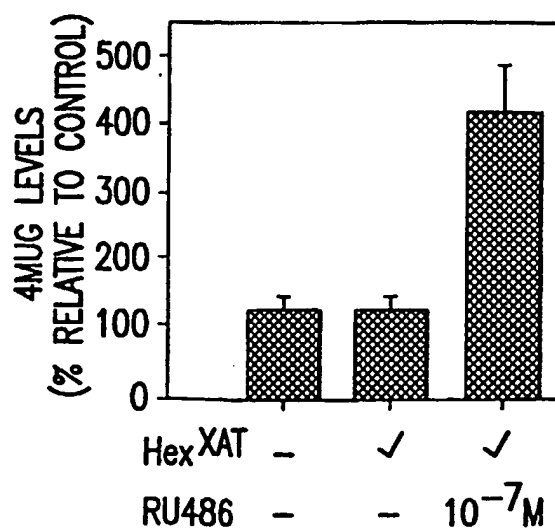
FIG.17B
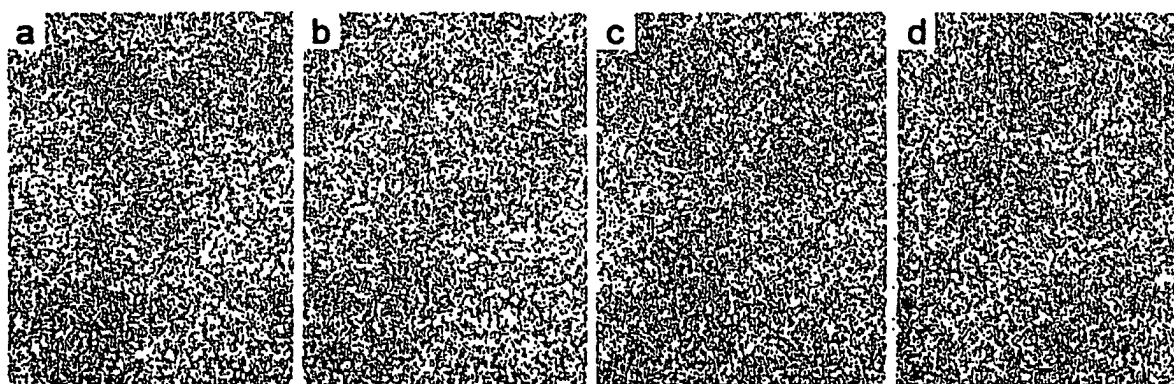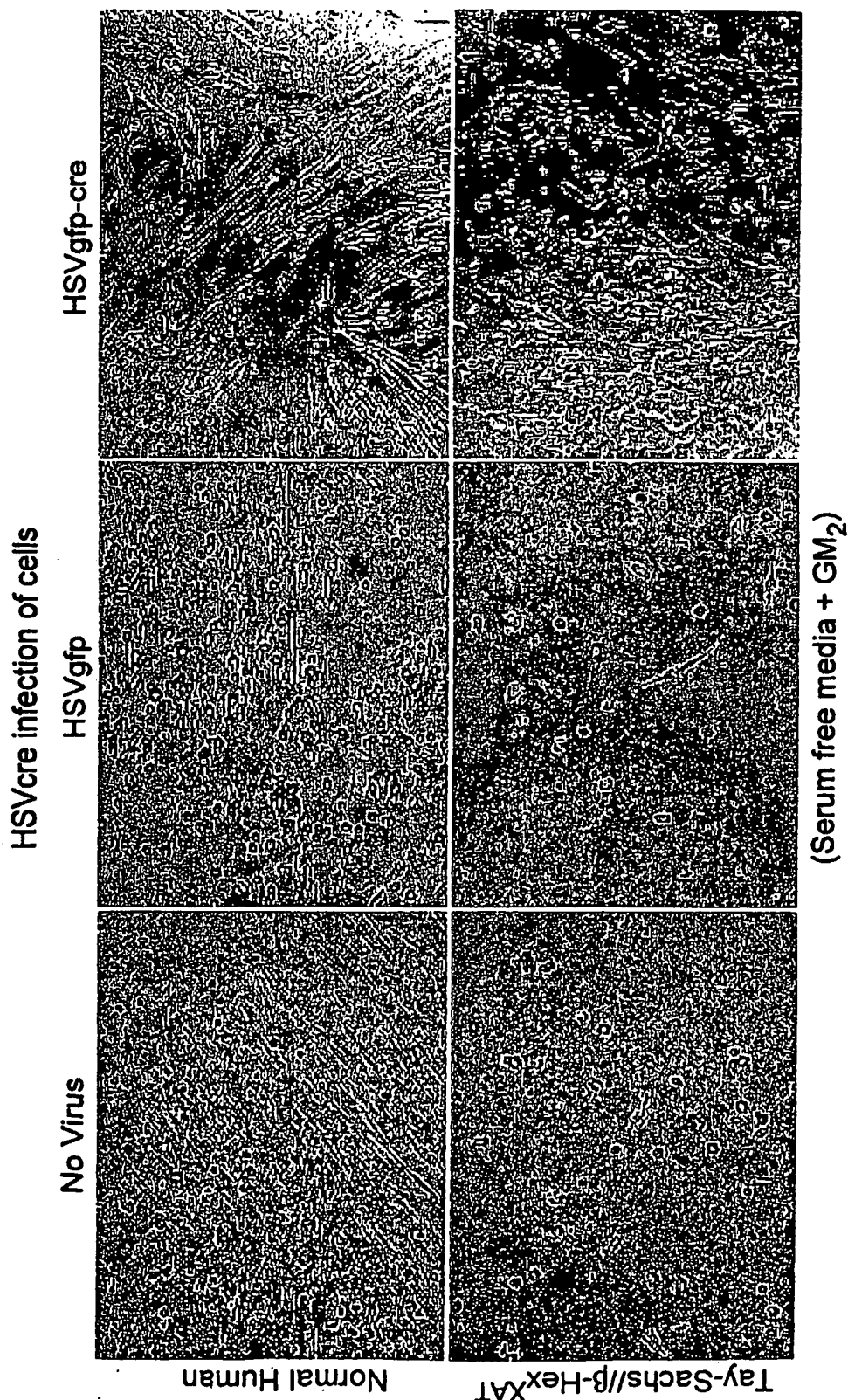
SUBSTITUTE SHEET (RULE 26)

FIG.17C

FIG. 18

(Serum free media + GM$_2$)
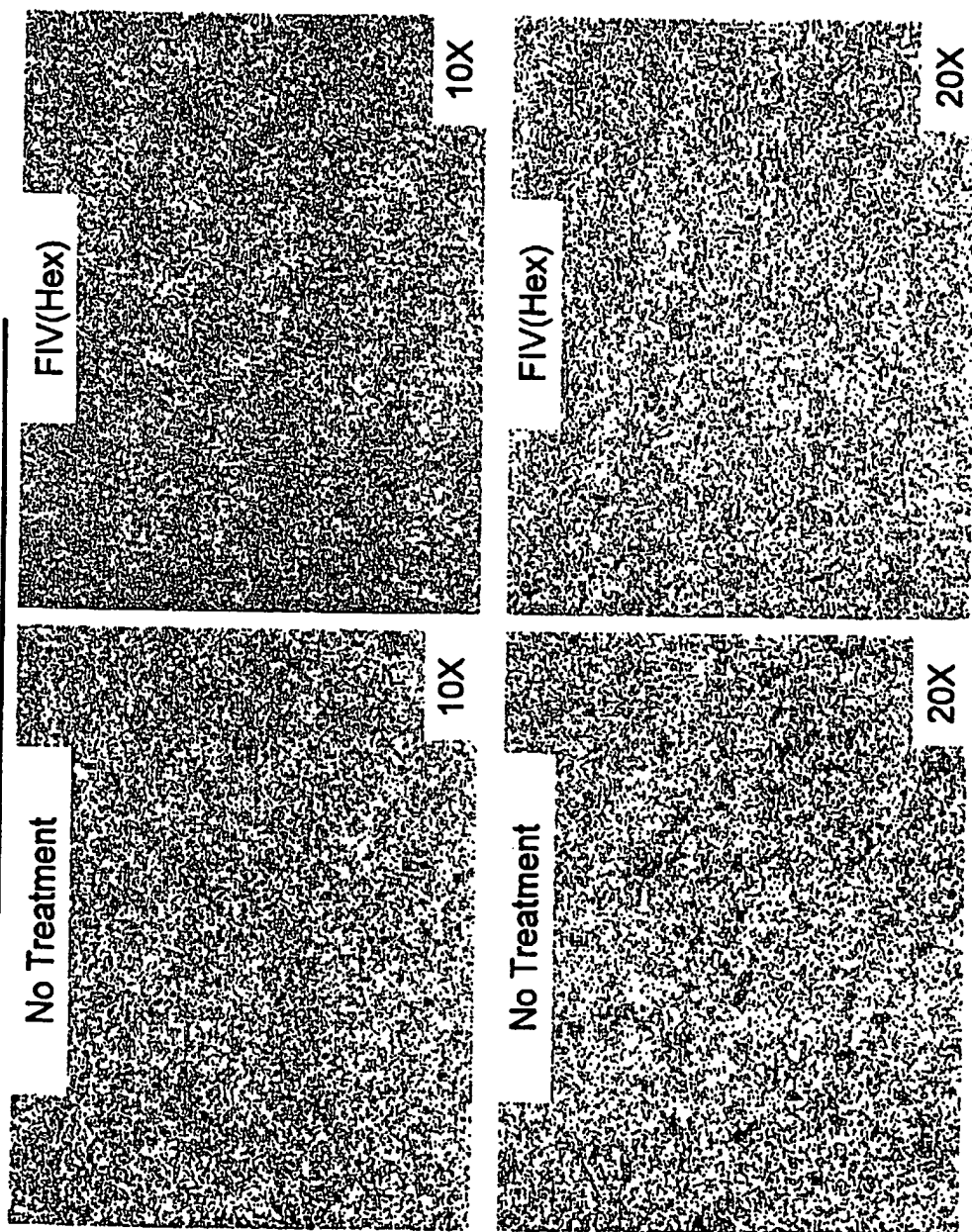
FIG.19
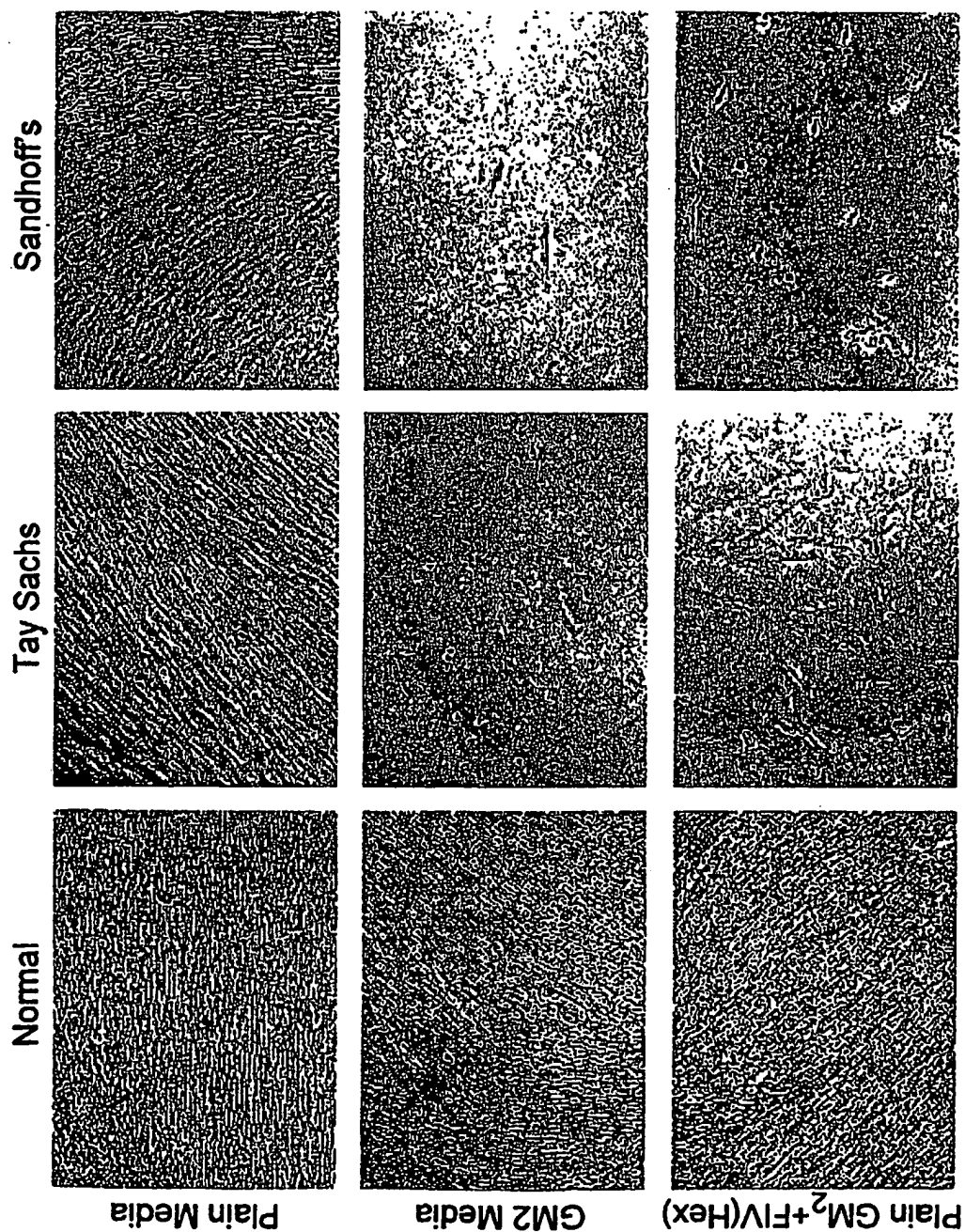
FIG.20

PERINATAL GENE THERAPY IN hexB$^{-/-}$ MICE

P2

FIV(Hex)
OR
FIV(lacZ)

13 14 15 16 17 ...

hexB$^{-/-}$
X
hexB$^{-/-}$

FIG.21

Perinatal FIV(Hex) in hexB-/-

Purkinje cells   40X

4th ventricle   100X

liver   40X

liver   100X

5 wks old

FIG.22

FIG.23

SEQUENCE LISTING

<110> University of Rochester
      Kyrkanides, Stephanos

<120> VECTORS HAVING BOTH ISOFORMS OF
  BETA-HEXOSAMINIDASE

<130> 21108.0018P1

<150> 60/377,503
<151> 2002-05-02

<160> 41

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 409
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 1
Met Met Thr Ser Val Tyr Ser Ser Leu Arg Leu Ser Gly Glu Leu Ser
 1   5   10   15
Glu Val Trp Arg Leu Leu Ala Ser Leu Phe Gly Asn Leu Leu Arg Ala
            20   25   30
Gln Phe Phe Ile Asn Lys Thr Glu Ile Glu Asp Phe Pro Arg Phe Pro
        35   40   45
His Arg Gly Leu Leu Leu Asp Thr Ser Arg His Tyr Leu Pro Leu Ser
    50   55   60
Ser Ile Leu Asp Thr Leu Asp Val Met Ala Tyr Asn Lys Leu Asn Val
65   70   75   80
Phe His Trp His Leu Val Asp Asp Pro Ser Phe Pro Tyr Glu Ser Phe
            85   90   95
Thr Phe Pro Glu Leu Met Arg Lys Gly Ser Tyr Asn Pro Val Thr His
            100   105   110
Ile Tyr Thr Ala Gln Asp Val Lys Glu Val Ile Glu Tyr Ala Arg Leu
            115   120   125
Arg Gly Ile Arg Val Leu Ala Glu Phe Asp Thr Pro Gly His Thr Leu
    130   135   140
Ser Trp Gly Pro Gly Ile Pro Gly Leu Leu Thr Pro Cys Tyr Ser Gly
145   150   155   160
Ser Glu Pro Ser Gly Thr Phe Gly Pro Val Asn Pro Ser Leu Asn Asn
                165   170   175
Thr Tyr Glu Phe Met Ser Thr Phe Phe Leu Glu Val Ser Ser Val Phe
            180   185   190
Pro Asp Phe Tyr Leu His Leu Gly Gly Asp Glu Val Asp Phe Thr Cys
            195   200   205
Trp Lys Ser Asn Pro Glu Ile Gln Asp Phe Met Arg Lys Lys Gly Phe
    210   215   220
Gly Glu Asp Phe Lys Gln Leu Glu Ser Phe Tyr Ile Gln Thr Leu Leu
225   230   235   240

```
Asp Ile Val Ser Ser Tyr Gly Lys Gly Tyr Val Val Trp Gln Glu Val
            245                     250                 255
Phe Asp Asn Lys Val Lys Ile Gln Pro Asp Thr Ile Ile Gln Val Trp
            260                     265                 270
Arg Glu Asp Ile Pro Val Asn Tyr Met Lys Glu Leu Glu Leu Val Thr
            275                     280                 285
Lys Ala Gly Phe Arg Ala Leu Leu Ser Ala Pro Trp Tyr Leu Asn Arg
    290                     295                 300
Ile Ser Tyr Gly Pro Asp Trp Lys Asp Phe Tyr Ile Val Glu Pro Leu
305                     310                 315                 320
Ala Phe Glu Gly Thr Pro Glu Gln Lys Ala Leu Val Ile Gly Gly Glu
                325                 330                 335
Ala Cys Met Trp Gly Glu Tyr Val Asp Asn Thr Asn Leu Val Pro Arg
            340                 345                 350
Leu Trp Pro Arg Ala Gly Ala Val Ala Glu Arg Leu Trp Ser Asn Lys
            355                 360                 365
Leu Thr Ser Asp Leu Thr Phe Ala Tyr Glu Arg Leu Ser His Phe Arg
    370                 375                 380
Cys Glu Leu Leu Arg Arg Gly Val Gln Ala Gln Pro Leu Asn Val Gly
385                 390                 395                 400
Phe Cys Glu Gln Glu Phe Glu Gln Thr
                405
```

<210> 2
<211> 2256
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 2
```
cctccgagag gggagaccag cgggccatga caagctccag gctttggttt tcgctgctgc        60
tggcggcagc gttcgcagga cgggcgacgg ccctctggcc ctggcctcag aacttccaaa       120
cctccgacca gcgctacgtc ctttacccga caactttca attccagtac gatgtcagct        180
cggccgcgca gcccggctgc tcagtcctcg acgaggcctt ccagcgctat cgtgacctgc       240
ttttcggttc cgggtcttgg ccccgtcctt acctcacagg gaaacggcat acactggaga       300
agaatgtgtt ggttgtctct gtagtcacac ctggatgtaa ccagcttcct actttggagt       360
cagtggagaa ttataccctg accataaatg atgaccagtg tttactcctc tctgagactg       420
tctggggagc tctccgaggt ctggagactt ttagccagct tgtttggaaa tctgctgagg       480
gcacagttct ttatcaacaa gactgagatt gaggactttc cccgctttcc tcaccggggc       540
ttgctgtggg atacatctcg ccattacctg ccactctcta gcatcctgga cactctggat       600
gtcatggcgt acaataaatt gaacggttc cactggcatc tggtagatga tccttccttc        660
ccatatgaga gcttcacttt tccagagctc atgagaaagg ggtccctacaa ccctgtcacc       720
cacatctaca cagcacagga tgtgaaggag gtcattgaat acgcacggct ccggggtatc       780
cgtgtgcttg cagagtttga cactcctggc cacactttgt cctggggacc aggtatccct       840
ggattactga ctccttgcta ctctgggtct gagccctctg gcacctttgg accagtgaat       900
cccagtctca ataatacta tgagttcatg agcacattct tcttagaagt cagctctgtc       960
ttcccagatt tttatcttca tcttggagga gatgaggttg atttcacctg ctggaagtcc      1020
aacccagaga tccaggactt tatgaggaag aaaggcttcg gtgaggactt caagcagctg      1080
gagtccttct acatccagac gctgctggac atcgtctctt cttatggcaa gggctatgtg      1140
gtgtggcagg aggtgtttga taataaagta aagattcagc cagacacaat catacaggtg      1200
tggcgagagg atattccagt gaactatatg aaggagctgg aactggtcac caaggccggc      1260
ttccgggccc ttctctctgc cccctggtac ctgaaccgta tatcctatgg ccctgactgg      1320
aaggatttct acatagtgga acccctggca tttgaaggta ccccctgagca gaaggctctg      1380
gtgattggtg gagaggcttg tatgtgggga gaatatgtgg acaacacaaa cctggtcccc      1440
aggctctggc ccagagcagg ggctgttgcc gaaaggctgt ggagcaacaa gttgacatct      1500
gacctgacat ttgcctatga acgtttgtca cacttccgct gtgaattgct gaggcgaggt      1560
```

```
gtccaggccc aacccctcaa tgtaggcttc tgtgagcagg agtttgaaca gacctgagcc    1620
ccaggcaccg aggagggtgc tggctgtagg tgaatggtag tggagccagg cttccactgc    1680
atcctggcca ggggacggag ccccttgcct tcgtgcccct tgcctgcgtg cccctgtgct    1740
tggagagaaa ggggccggtg ctggcgctcg cattcaataa agagtaatgt ggcattttc     1800
tataataaac atggattacc tgtgtttaaa aaaaaaagtg tgaatggcgt tagggtaagg    1860
gcacagccag gctggagtca gtgtctgccc ctgaggtctt ttaagttgag ggctgggaat    1920
gaaacctata gcctttgtgc tgttctgcct tgcctgtgag ctatgtcact cccctcccac    1980
tcctgaccat attccagaca cctgccctaa tcctcagcct gctcacttca cttctgcatt    2040
atatctccaa ggcgttggta tatggaaaaa gatgtagggg cttggaggtg ttctggacag    2100
tggggagggc tccagaccca acctggtcac agaagagcct ctcccccatg catactcatc    2160
cacctccctc ccctagagct attctccttt gggtttcttg ctgcttcaat tttatacaac    2220
cattatttaa atattattaa acacatattg ttctct                              2256
```

<210> 3
<211> 544
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
     Synthetic Construct

<400> 3
```
Met Leu Leu Ala Leu Leu Leu Ala Thr Leu Leu Ala Ala Met Leu Ala
 1               5                   10                  15
Leu Leu Thr Gln Val Ala Leu Val Val Gln Val Ala Glu Ala Ala Arg
            20                  25                  30
Ala Pro Ser Val Ser Ala Lys Pro Gly Pro Ala Leu Trp Pro Leu Pro
        35                  40                  45
Leu Leu Val Lys Met Thr Pro Asn Leu Leu His Leu Ala Pro Glu Asn
    50                  55                  60
Phe Tyr Ile Ser His Ser Pro Asn Ser Thr Ala Gly Pro Ser Cys Thr
65                  70                  75                  80
Leu Leu Glu Glu Ala Phe Arg Arg Tyr His Gly Tyr Ile Phe Gly Phe
                85                  90                  95
Tyr Lys Trp His His Glu Pro Ala Glu Phe Gln Ala Lys Thr Gln Val
            100                 105                 110
Gln Gln Leu Leu Val Ser Ile Thr Leu Gln Ser Glu Cys Asp Ala Phe
        115                 120                 125
Pro Asn Ile Ser Ser Asp Glu Ser Tyr Thr Leu Leu Val Lys Glu Pro
    130                 135                 140
Val Ala Val Leu Lys Ala Asn Arg Val Trp Gly Ala Leu Arg Gly Leu
145                 150                 155                 160
Glu Thr Phe Ser Gln Leu Val Tyr Gln Asp Ser Tyr Gly Thr Phe Thr
                165                 170                 175
Ile Asn Glu Ser Thr Ile Ile Asp Ser Pro Arg Phe Ser His Arg Gly
            180                 185                 190
Ile Leu Ile Asp Thr Ser Arg His Tyr Leu Pro Val Lys Ile Ile Leu
        195                 200                 205
Lys Thr Leu Asp Ala Met Ala Phe Asn Lys Phe Asn Val Leu His Trp
    210                 215                 220
His Ile Val Asp Asp Gln Ser Phe Pro Tyr Gln Ser Ile Thr Phe Pro
225                 230                 235                 240
Glu Leu Ser Asn Lys Gly Ser Tyr Ser Leu Ser His Val Tyr Thr Pro
                245                 250                 255
Asn Asp Val Arg Met Val Ile Glu Tyr Ala Arg Leu Arg Gly Ile Arg
            260                 265                 270
Val Leu Pro Glu Phe Asp Thr Pro Gly His Thr Leu Ser Trp Gly Lys
        275                 280                 285
```

```
Gly Gln Lys Asp Leu Leu Thr Pro Cys Tyr Ser Arg Gln Asn Lys Leu
    290                     295                 300
Asp Ser Phe Gly Pro Ile Asn Pro Thr Leu Asn Thr Thr Tyr Ser Phe
305                     310                 315                 320
Leu Thr Thr Phe Phe Lys Glu Ile Ser Glu Val Phe Pro Asp Gln Phe
                325                 330                 335
Ile His Leu Gly Gly Asp Glu Val Glu Phe Lys Cys Trp Glu Ser Asn
            340                 345                 350
Pro Lys Ile Gln Asp Phe Met Arg Gln Lys Gly Phe Gly Thr Asp Phe
        355                 360                 365
Lys Lys Leu Glu Ser Phe Tyr Ile Gln Lys Val Leu Asp Ile Ile Ala
    370                 375                 380
Thr Ile Asn Lys Gly Ser Ile Val Trp Gln Glu Val Phe Asp Asp Lys
385                 390                 395                 400
Ala Lys Leu Ala Pro Gly Thr Ile Val Glu Val Trp Lys Asp Ser Ala
                405                 410                 415
Tyr Pro Glu Glu Leu Ser Arg Val Thr Ala Ser Gly Phe Pro Val Ile
            420                 425                 430
Leu Ser Ala Pro Trp Tyr Leu Asp Leu Ile Ser Tyr Gly Gln Asp Trp
            435                 440                 445
Arg Lys Tyr Tyr Lys Val Glu Pro Leu Asp Phe Gly Gly Thr Gln Lys
    450                 455                 460
Gln Lys Gln Leu Phe Ile Gly Gly Glu Ala Cys Leu Trp Gly Glu Tyr
465                 470                 475                 480
Val Asp Ala Thr Asn Leu Thr Pro Arg Leu Trp Pro Arg Ala Ser Ala
                485                 490                 495
Val Gly Glu Arg Leu Trp Ser Ser Lys Asp Val Arg Asp Met Asp Asp
            500                 505                 510
Ala Tyr Asp Arg Leu Thr Arg His Arg Cys Arg Met Val Glu Arg Gly
        515                 520                 525
Ile Ala Ala Gln Pro Leu Tyr Ala Gly Tyr Cys Asn His Glu Asn Met
    530                 535                 540
```

```
<210> 4
<211> 1635
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 4
atgctgctgg cgctgctgtt ggcgacactg ctggcggcga tgttggcgct gctgactcag      60
gtggcgctgg tggtgcaggt ggcggaggcg gctcgggccc cgagcgtctc ggccaagccg     120
gggccggcgc tgtggcccct gccgctcttg gtgaagatga ccccgaacct gctgcatctc     180
gccccggaga acttctacat cagccacagc cccaattcca cggcgggccc ctcctgcacc     240
ctgctggagg aagcgtttcg acgatatcat ggctatattt ttggtttcta caagtggcat     300
catgaacctg ctgaattcca ggctaaaacc caggttcagc aacttcttgt ctcaatcacc     360
cttcagtcag agtgtgatgc tttccccaac atatcttcag atgagtctta tactttactt     420
gtgaaagaac cagtggctgt ccttaaggcc aacagagttt ggggagcatt acgaggttta     480
gagaccttta gccagttagt ttatcaagat tcttatggaa ctttcaccat caatgaatcc     540
accattattg attctccaag gtttttctca cagaggaattt tgattgatac atccagacat     600
tatctgccag ttaagattat tcttaaaact ctggatgcca tggctttttaa taagtttaat     660
gttcttcact ggcacatagt tgatgaccag tctttcccat atcagagcat cacttttcct     720
gagttaagca ataaaggaag ctattctttg tctcatgttt atacaccaaa tgatgtccgt     780
atggtgattg aatatgccag attacgagga attcgagtcc tgccagaatt tgatacccct     840
gggcatacac tatcttgggg aaaaggtcag aaagacctcc tgactccatg ttacagtaga     900
caaaacaagt tggactcttt tggacctata aaccctactc tgaatacaac atacagcttc     960
```

```
cttactacat ttttcaaaga aattagtgag gtgtttccag atcaattcat tcatttggga   1020
ggagatgaag tggaatttaa atgttgggaa tcaaatccaa aaattcaaga tttcatgagg   1080
caaaaaggct ttggcacaga tttttaagaaa ctagaatctt tctacattca aaaggttttg   1140
gatattattg caaccataaa caagggatcc attgtctggc aggaggtttt tgatgataaa   1200
gcaaagcttg cgccgggcac aatagttgaa gtatggaaag acagcgcata tcctgaggaa   1260
ctcagtagag tcacagcatc tggcttccct gtaatccttt ctgctccttg gtacttagat   1320
ttgattagct atggacaaga ttggaggaaa tactataaag tggaacctct tgattttggc   1380
ggtactcaga aacagaaaca acttttcatt ggtggagaag cttgtctatg gggagaatat   1440
gtggatgcaa ctaacctcac tccaagatta tggcctcggg caagtgctgt tggtgagaga   1500
ctctggagtt ccaaagatgt cagagatatg gatgacgcct atgacagact gacaaggcac   1560
cgctgcagga tggtcgaacg tggaatagct gcacaacctc tttatgctgg atattgtaac   1620
catgagaaca tgtaa                                                    1635
```

<210> 5
<211> 581
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 5
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aattccgccc ctctccctcc cccccccta acgttactgg ccgaagccgc ttggaataag    60
gccggtgtgc gtttgtctat atgtgatttt ccaccatatt gccgtctttt ggcaatgtga   120
gggcccggaa acctggcccт gtcttcttga cgagcattcc taggggtctt tcccctctcg   180
ccaaaggaat gcaaggtctg ttgaatgtcg tgaaggaagc agttcctctg gaagcttctt   240
gaagacaaac aacgtctgta gcgacccttt gcaggcagcg gaaccccca cctggcgaca   300
ggtgcctctg cggccaaaag ccacgtgtat aagatacacc tgcaaaggcg gcacaaccc   360
agtgccacgt tgtgagttgg atagttgtgg aaagagtcaa atggctctcc tcaagcgtat   420
tcaacaaggg gctgaaggat gcccagaagg taccccattg tatgggatct gatctggggc   480
ctcggtgcac atgctttaca tgtgtttagt cgaggttaaa aaaacgtcta ggccccccga   540
accacgggga cgtggttttc ctttgaaaaa cacgatgata a                       581
```

<210> 6
<211> 528
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 6
```
Met Ala Gly Cys Arg Leu Trp Val Ser Leu Leu Leu Ala Ala Ala Leu
1               5                   10                  15
Ala Cys Leu Ala Thr Ala Leu Trp Pro Trp Pro Gln Tyr Ile Gln Thr
            20                  25                  30
Tyr His Arg Arg Tyr Thr Leu Tyr Pro Asn Asn Phe Gln Phe Arg Tyr
        35                  40                  45
His Val Ser Ser Ala Ala Gln Gly Gly Cys Val Val Leu Asp Glu Ala
    50                  55                  60
Phe Arg Arg Tyr Arg Asn Leu Leu Phe Gly Ser Gly Ser Trp Pro Arg
65                  70                  75                  80
Pro Ser Phe Ser Asn Lys Gln Gln Thr Leu Gly Lys Asn Ile Leu Val
            85                  90                  95
Val Ser Val Val Thr Ala Glu Cys Asn Glu Phe Pro Asn Leu Glu Ser
            100                 105                 110
```

```
Val Glu Asn Tyr Thr Leu Thr Ile Asn Asp Asp Gln Cys Leu Leu Ala
        115                 120                 125
Ser Glu Thr Val Trp Gly Ala Leu Arg Gly Leu Glu Thr Phe Ser Gln
    130                 135                 140
Leu Val Trp Lys Ser Ala Glu Gly Thr Phe Phe Ile Asn Lys Thr Lys
145                 150                 155                 160
Ile Lys Asp Phe Pro Arg Phe Pro His Arg Gly Val Leu Leu Asp Thr
                165                 170                 175
Ser Arg His Tyr Leu Pro Leu Ser Ser Ile Leu Asp Thr Leu Asp Val
                180                 185                 190
Met Ala Tyr Asn Lys Phe Asn Val Phe His Trp His Leu Val Asp Asp
            195                 200                 205
Ser Ser Phe Pro Tyr Glu Ser Phe Thr Phe Pro Glu Leu Thr Arg Lys
    210                 215                 220
Gly Ser Phe Asn Pro Val Thr His Ile Tyr Thr Ala Gln Asp Val Lys
225                 230                 235                 240
Glu Val Ile Glu Tyr Ala Arg Leu Arg Gly Ile Arg Val Leu Ala Glu
                245                 250                 255
Phe Asp Thr Pro Gly His Thr Leu Ser Trp Gly Pro Gly Ala Pro Gly
            260                 265                 270
Leu Leu Thr Pro Cys Tyr Ser Gly Ser His Leu Ser Gly Thr Phe Gly
        275                 280                 285
Pro Val Asn Pro Ser Leu Asn Ser Thr Tyr Asp Phe Met Ser Thr Leu
    290                 295                 300
Phe Leu Glu Ile Ser Ser Val Phe Pro Asp Phe Tyr Leu His Leu Gly
305                 310                 315                 320
Gly Asp Glu Val Asp Phe Thr Cys Trp Lys Ser Asn Pro Asn Ile Gln
                325                 330                 335
Ala Phe Met Lys Lys Lys Gly Phe Thr Asp Phe Lys Gln Leu Glu Ser
            340                 345                 350
Phe Tyr Ile Gln Thr Leu Leu Asp Ile Val Ser Asp Tyr Asp Lys Gly
        355                 360                 365
Tyr Val Val Trp Gln Glu Val Phe Asp Asn Lys Val Lys Val Arg Pro
    370                 375                 380
Asp Thr Ile Ile Gln Val Trp Arg Glu Glu Met Pro Val Glu Tyr Met
385                 390                 395                 400
Leu Glu Met Gln Asp Ile Thr Arg Ala Gly Phe Arg Ala Leu Leu Ser
                405                 410                 415
Ala Pro Trp Tyr Leu Asn Arg Val Lys Tyr Gly Pro Asp Trp Lys Asp
            420                 425                 430
Met Tyr Lys Val Glu Pro Leu Ala Phe His Gly Thr Pro Glu Gln Lys
        435                 440                 445
Ala Leu Val Ile Gly Gly Glu Ala Cys Met Trp Gly Glu Tyr Val Asp
    450                 455                 460
Ser Thr Asn Leu Val Pro Arg Leu Trp Pro Arg Ala Gly Ala Val Ala
465                 470                 475                 480
Glu Arg Leu Trp Ser Ser Asn Leu Thr Thr Asn Ile Asp Phe Ala Phe
                485                 490                 495
Lys Arg Leu Ser His Phe Arg Cys Glu Leu Val Arg Arg Gly Ile Gln
                500                 505                 510
Ala Gln Pro Ile Ser Val Gly Tyr Cys Glu Gln Glu Phe Glu Gln Thr
        515                 520                 525
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<210> 7
<211> 1960
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 7
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gcctgctggc cggggagctg gccggtgggc atggccggct gcaggctctg ggtttcgctg     180
ctgctggcgg cggcgttggc ttgcttggcc acggcactgt ggccgtggcc ccagtacatc     240
caaacctacc accggcgcta caccctgtac cccaacaact tccagttccg gtaccatgtc     300
agttcggccg cgcagggcgg ctgcgtcgtc ctcgacgagg cctttcgacg ctaccgtaac     360
ctgctcttcg gttccggctc ttggccccga cccagcttct caaataaaca gcaaacgttg     420
gggaagaaca ttctggtggt ctccgtcgtc acagctgaat gtaatgaatt tcctaatttg     480
gagtcggtag aaaattacac cctaaccatt aatgatgacc agtgtttact cgcctctgag     540
actgtctggg gcgctctccg aggtctggag actttcagtc agcttgtttg gaaatcagct     600
gagggcacgt tctttatcaa caagacaaag attaaagact ttcctcgatt ccctcaccgg     660

ggcgtactgc tggatacatc tcgccattac ctgccattgt ctagcatcct ggatacactg     720
gatgtcatgg catacaataa attcaacgtg ttccactggc acttggtgga cgactcttcc     780
ttcccatatg agagcttcac tttcccagag ctcaccagaa aggggtcctt caaccctgtc     840
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aacccccagtc tcaacagcac ctatgacttc atgagcacac tcttcctgga gatcagctca   1080
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gagtccttct acatccagac gctgctggac atcgtctctg attatgacaa gggctatgtg    1260
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aatatagact ttgcctttaa acgtttgtcg catttccgtt gtgagctggt gaggagagga    1680
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agggtgctgg ccttaagaga gcaggtcccg gggcagggct aatctttcac tgcctcccgg    1860
ccaggggaga gcaccccttg cccgtgtgcc cctgtgacta cagagaagga ggctggtgct    1920
ggcactggtg ttcaataaag atctatgtgg cattttctct                          1960
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<210> 8
<211> 12745
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 8
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ccaaaatgtc gtaacaactc cgccccattg acgcaaatgg gcggtaggcg tgtacggtgg     180
gaggtctata taagcagagc tctgtgaaac ttcgaggagt ctctttgttg aggacttttg     240
agttctccct tgaggctccc acagatacaa taaatatttg agattgaacc ctgtcgagta     300
tctgtgtaat cttttttacc tgtgaggtct cggaatccgg gccgagaact tcgcagttgg     360
cgcccgaaca gggacttgat tgagagtgat tgaggaagtg aagctagagc aatagaaagc     420
tgttaagcag aactcctgct gacctaaata gggaagcagt agcagacgct gctaacagtg     480
agtatctcta gtgaagcgga ctcgagctca taatcaagtc attgtttaaa ggcccagata     540
```

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aattacatct ggtgactctt cgcggacctt caagccagga gattcgccga gggacagtca       600

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gccattaaga gatgtagtaa tgttgctgta ggagtagggg ggaagagtaa aaaatttgga       720
gaagggaatt tcagatgggc cattagaatg gctaatgtat ctacaggacg agaacctggt       780
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tccacaggca tatcctattc aaacagtaaa tggagtacca caatatgtag cacttgaccc      1080
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gctacactag aaggacagta tttggtatct gcgctctgct gaagccagtt accttcggaa   10860
aaagagttgg tagctcttga tccggcaaac aaaccaccgc tggtagcggt ggtttttttg   10920
tttgcaagca gcagattacg cgcagaaaaa aaggatctca agaagatcct ttgatctttt   10980
ctacggggtc tgacgctcag tggaacgaaa actcacgtta agggattttg gtcatgagat   11040
tatcaaaaag gatcttcacc tagatccttt taaattaaaa atgaagtttt aaatcaatct   11100
```

```
aaagtatata tgagtaaact tggtctgaca gttaccaatg cttaatcagt gaggcaccta    11160
tctcagcgat ctgtctattt cgttcatcca tagttgcctg actccccgtc gtgtagataa    11220
ctacgatacg ggagggctta ccatctggcc ccagtgctgc aatgataccg cgagacccac    11280
gctcaccggc tccagatta tcagcaataa accagccagc cggaagggcc gagcgcagaa    11340
gtggtcctgc aactttatcc gcctccatcc agtctattaa ttgttgccgg gaagctagag    11400
taagtagttc gccagttaat agtttgcgca acgttgttgc cattgctaca ggcatcgtgg    11460
tgtcacgctc gtcgtttggt atggcttcat tcagctccgg ttcccaacga tcaaggcgag    11520
ttacatgatc ccccatgttg tgcaaaaaag cggttagctc cttcggtcct ccgatcgttg    11580
tcagaagtaa gttggccgca gtgttatcac tcatggttat ggcagcactg cataattctc    11640
ttactgtcat gccatccgta agatgctttt ctgtgactgg tgagtactca accaagtcat    11700
tctgagaata gtgtatgcgg cgaccgagtt gctcttgccc ggcgtcaata cgggataata    11760
ccgcgccaca tagcagaact ttaaaagtgc tcatcattgg aaaacgttct cgggggcgaa    11820
aactctcaag gatcttaccg ctgttgagat ccagttcgat gtaacccact cgtgcaccca    11880
actgatcttc agcatctttt actttcacca gcgtttctgg gtgagcaaaa acaggaaggc    11940
aaaatgccgc aaaaaaggga ataagggcga cacggaaatg ttgaatactc atactcttcc    12000
tttttcaata ttattgaagc atttatcagg gttattgtct catgagcgga tacatatttg    12060
aatgtattta gaaaaataaa caaataggg ttccgcgcac atttccccga aaagtgccac    12120
ctgacgtcga cggatcggga gatctcccga tcccctatgg tcgactctca gtacaatctg    12180
ctctgatgcc gcatagttaa gccagtatct gctccctgct tgtgtgttgg aggtcgctga    12240
gtagtgcgcg agcaaaattt aagctacaac aaggcaaggc ttgaccgaca attgcatgaa    12300
gaatctgctt agggttaggc gttttgcgct gcttcgcgat gtacgggcca gatatacgcg    12360
ttgacattga ttattgacta gttattaata gtaatcaatt acggggtcat tagttcatag    12420
cccatatatg gagttccgcg ttacataact tacggtaaat ggcccgcctg gctgaccgcc    12480
caacgacccc cgcccattga cgtcaataat gacgtatgtt cccatagtaa cgccaatagg    12540
gactttccat tgacgtcaat gggtggacta tttacggtaa actgcccact tggcagtaca    12600
tcaagtgtat catatgccaa gtacgccccc tattgacgtc aatgacggta aatggcccgc    12660
ctggcattat gcccagtaca tgaccttatg ggactttcct acttggcagt acatctacgt    12720
attagtcatc gctattacca tggtg                                        12745
```

```
<210> 9
<211> 529
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 9
Met Thr Ser Ser Arg Leu Trp Phe Ser Leu Leu Leu Ala Ala Ala Phe
1               5                   10                  15
Ala Gly Arg Ala Thr Ala Leu Trp Pro Trp Pro Gln Asn Phe Gln Thr
            20                  25                  30
Ser Asp Gln Arg Tyr Val Leu Tyr Pro Asn Asn Phe Gln Phe Gln Tyr
        35                  40                  45
Asp Val Ser Ser Ala Ala Gln Pro Gly Cys Ser Val Leu Asp Glu Ala
    50                  55                  60
Phe Gln Arg Tyr Arg Asp Leu Leu Phe Gly Ser Gly Ser Trp Pro Arg
65                  70                  75                  80
Pro Tyr Leu Thr Gly Lys Arg His Thr Leu Glu Lys Asn Val Leu Val
                85                  90                  95
Val Ser Val Val Thr Pro Gly Cys Asn Gln Leu Pro Thr Leu Glu Ser
            100                 105                 110
Val Glu Asn Tyr Thr Leu Thr Ile Asn Asp Asp Gln Cys Leu Leu Leu
        115                 120                 125
Ser Glu Thr Val Trp Gly Ala Leu Arg Gly Leu Glu Thr Phe Ser Gln
    130                 135                 140
```

Leu Val Trp Lys Ser Ala Glu Gly Thr Phe Phe Ile Asn Lys Thr Glu
145   150   155   160

Ile Glu Asp Phe Pro Arg Phe Pro His Arg Gly Leu Leu Leu Asp Thr
                165   170   175

Ser Arg His Tyr Leu Pro Leu Ser Ser Ile Leu Asp Thr Leu Asp Val
            180   185   190

Met Ala Tyr Asn Lys Leu Asn Val Phe His Trp His Leu Val Asp Asp
            195   200   205

Pro Ser Phe Pro Tyr Glu Ser Phe Thr Phe Pro Glu Leu Met Arg Lys
            210   215   220

Gly Ser Tyr Asn Pro Val Thr His Ile Tyr Thr Ala Gln Asp Val Lys
225   230   235   240

Glu Val Ile Glu Tyr Ala Arg Leu Arg Gly Ile Arg Val Leu Ala Glu
                245   250   255

Phe Asp Thr Pro Gly His Thr Leu Ser Trp Gly Pro Gly Ile Pro Gly
            260   265   270

Leu Leu Thr Pro Cys Tyr Ser Gly Ser Glu Pro Ser Gly Thr Phe Gly
            275   280   285

Pro Val Asn Pro Ser Leu Asn Asn Thr Tyr Glu Phe Met Ser Thr Phe
290   295   300

Phe Leu Glu Val Ser Ser Val Phe Pro Asp Phe Tyr Leu His Leu Gly
305   310   315   320

Gly Asp Glu Val Asp Phe Thr Cys Trp Lys Ser Asn Pro Glu Ile Gln
            325   330   335

Asp Phe Met Arg Lys Lys Gly Phe Gly Glu Asp Phe Lys Gln Leu Glu
            340   345   350

Ser Phe Tyr Ile Gln Thr Leu Leu Asp Ile Val Ser Ser Tyr Gly Lys
            355   360   365

Gly Tyr Val Val Trp Gln Glu Val Phe Asp Asn Lys Val Lys Ile Gln
370   375   380

Pro Asp Thr Ile Ile Gln Val Trp Arg Glu Asp Ile Pro Val Asn Tyr
385   390   395   400

Met Lys Glu Leu Glu Leu Val Thr Lys Ala Gly Phe Arg Ala Leu Leu
            405   410   415

Ser Ala Pro Trp Tyr Leu Asn Arg Ile Ser Tyr Gly Pro Asp Trp Lys
            420   425   430

Asp Phe Tyr Val Val Glu Pro Leu Ala Phe Glu Gly Thr Pro Glu Gln
            435   440   445

Lys Ala Leu Val Ile Gly Gly Glu Ala Cys Met Trp Gly Glu Tyr Val
450   455   460

Asp Asn Thr Asn Leu Val Pro Arg Leu Trp Pro Arg Ala Gly Ala Val
465   470   475   480

Ala Glu Arg Leu Trp Ser Asn Lys Leu Thr Ser Asp Leu Thr Phe Ala
            485   490   495

Tyr Glu Arg Leu Ser His Phe Arg Cys Glu Leu Leu Arg Arg Gly Val
            500   505   510

Gln Ala Gln Pro Leu Asn Val Gly Phe Cys Glu Gln Glu Phe Glu Gln
            515   520   525

Thr

<210> 10
<211> 2255
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 10

```
cctccgagag gggagaccag cgggccatga caagctccag gctttggttt tcgctgctgc    60
tggcggcagc gttcgcagga cgggcgacgg ccctctggcc ctggcctcag aacttccaaa   120
cctccgacca gcgctacgtc ctttacccga caactttca attccagtac gatgtcagct    180
cggccgcgca gcccggctgc tcagtcctcg acgaggcctt ccagcgctat cgtgacctgc   240
tttcggttc cgggtcttgg ccccgtcctt acctcacagg gaaacggcat acactggaga    300
agaatgtgtt ggttgtctct gtagtcacac ctggatgtaa ccagcttcct actttggagt    360
cagtggagaa ttatacctg accataaatg atgaccagtg tttactcctc tctgagactg      420
tctggggagc tctccgaggt ctggagactt ttagccagct tgtttggaaa tctgctgagg    480
gcacattctt tatcaacaag actgagattg aggactttcc ccgctttcct caccgggggct   540
tgctgttgga tacatctcgc cattacctgc cactctctag catcctggac actctggatg     600
tcatggcgta caataaattg aacgtgttcc actggcatct ggtagatgat ccttccttcc    660
catatgagag cttcactttt ccagagctca tgagaaaggg gtcctacaac cctgtcaccc    720
acatctacac agcacaggat gtgaaggagg tcattgaata cgcacggctc cggggtatcc    780
gtgtgcttgc agagtttgac actcctggcc acactttgtc ctggggacca ggtatccctg      840
gattactgac tccttgctac tctgggtctg agccctctgg cacctttgga ccagtgaatc     900
ccagtctcaa taatacctat gagttcatga gcacattctt cttagaagtc agctctgtct     960
tcccagattt ttatcttcat cttggaggag atgaggttga tttcacctgc tggaagtcca   1020
acccagagat ccaggacttt atgaggaaga aaggcttcgg tgaggacttc aagcagctgg   1080
agtccttcta catccagacg ctgctggaca tcgtctcttc ttatggcaag ggctatgtgg   1140
tgtggcagga ggtgtttgat aataaagtaa agattcagcc agacacaatc atacaggtgt  1200
ggcgagagga tattccagtg aactatatga aggagctgga actggtcacc aaggccggct  1260
tccgggccct tctctctgcc ccctggtacc tgaaccgtat atcctatggc cctgactgga   1320
aggatttcta cgtagtggaa ccctggcat ttgaaggtac ccctgagcag aaggctctgg   1380
tgattggtgg agaggcttgt atgtggggag aatatgtgga caacacaaac ctggtccca    1440
ggctctggcc cagagcaggg gctgttgccg aaaggctgtg gagcaacaag ttgacatctg   1500
acctgacatt tgcctatgaa cgtttgtcac acttccgctg tgagttgctg aggcgaggtg  1560
tccaggccca acccctcaat gtaggcttct gtgagcagga gtttgaacag acctgagccc   1620
caggcaccga ggagggtgct ggctgtaggt gaatggtagt ggagccaggc ttccactgca  1680
tcctggccag gggacggagc cccttgcctt cgtgcccctt gcctgcgtgc ccctgtgctt   1740
ggagagaaag gggccggtgc tggcgctcgc attcaataaa gagtaatgtg gcatttttct  1800
ataataaaca tggattacct gtgtttaaaa aaaaagtgt gaatggcgtt agggtaaggg   1860
cacagccagg ctggagtcag tgtctgcccc tgaggtcttt taagttgagg gctgggaatg   1920
aaacctatg cctttgtgct gttctgcctt gcctgtgagc tatgtcactc ccctcccact    1980
cctgaccata ttccagacac ctgccctaat cctcagcctg ctcacttcac ttctgcatta   2040
tatctccaag gcgttggtat atggaaaaag atgtaggggc ttggaggtgt tctggacagt   2100
ggggagggct ccagacccaa cctggtcaca aaagagcctc tcccccatgc atactcatcc  2160
acctccctcc cctagagcta ttctcctttg ggtttcttgc tgctgcaatt ttatacaacc   2220
attatttaaa tattattaaa cacatattgt tctct                             2255
```

<210> 11
<211> 1635
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 11

```
atgctactgg cgctgctgtt ggcgacactg ctggcggcga tgttggcgct gctgactcag    60
gtggcgctgg tggtgcaggt ggcggaggcg gctcgggccc cgagcgtctc ggccaagccg   120
gggccggcgc tgtggcccct gccgctcttg gtgaagatga ccccgaacct gctgcatctc    180
gccccggaga acttctacat cagccacagc cccaattcca cggcgggccc ctcctgcacc    240
ctgctggagg aagcgtttcg acgatatcat ggctatattt ttggtttcta caagtggcat    300
catgaacctg ctgaattcca ggctaaaacc caggttcagc aacttcttgt ctcaatcacc   360
```

```
cttcagtcag agtgtgatgc tttccccaac atatcttcag atgagtctta tactttactt    420
gtgaaagaac cagtggctgt ccttaaggcc aacagagttt ggggagcatt acgaggttta    480
gagacctta gccagttagt ttatcaagat tcttatggaa ctttcaccat caatgaatcc    540
accattattg attctccaag gttttctcac agaggaattt tgattgatac atccagacat    600
tatctgccag ttaagattat tcttaaaact ctggatgcca tggctttaa taagtttaat    660
gttcttcact ggcacatagt tgatgaccag tctttcccat atcagagcat cacttttcct    720
gagttaagca ataaaggaag ctattctttg tctcatgttt atacaccaaa tgatgtccgt    780
atggtgattg aatatgccag attacgagga attcgagtcc tgccagaatt tgataccct    840
gggcatacac tatcttgggg aaaaggtcag aaagacctcc tgactccatg ttacagtaga    900
caaaacaagt tggactcttt tggacctata aaccctactc tgaatacaac atacagcttc    960
cttactacat ttttcaaaga aattagtgag gtgtttccag atcaattcat tcatttggga   1020
ggagatgaag tggaatttaa atgttgggaa tcaaatccaa aaattcaaga tttcatgagg   1080
caaaaaggct ttggcacaga ttttaagaaa ctagaatctt tctacattca aaaggttttg   1140
gatattattg caaccataaa caaggatcc attgtctggc aggaggtttt tgatgataaa   1200
gcaaagcttg cgccgggcac aatagttgaa gtatggaaag acagcgcata tcctgaggaa   1260
ctcagtagag tcacagcatc tggcttccct gtaatccttt ctgctccttg gtacttagat   1320
ttgattagct atggacaaga ttggaggaaa tactataaag tggaacctct tgattttggc   1380
ggtactcaga aacagaaaca acttttcatt ggtggagaag cttgtctatg gggagaatat   1440
gtggatgcaa ctaacctcac tccaagatta tggcctcggg caagtgctgt tggtgagaga   1500
ctctggagtt ccaaagatgt cagagatatg gatgacgcct atgacagact gacaaggcac   1560
cgctgcagga tggtcgaacg tggaatagct gcacaacctc tttatgctgg atattgtaac   1620
catgagaaca tgtaa                                                   1635
```

<210> 12
<211> 544
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 12
```
Met Leu Leu Ala Leu Leu Leu Ala Thr Leu Leu Ala Ala Met Leu Ala
1               5                   10                  15
Leu Leu Thr Gln Ile Ala Leu Val Val Gln Val Ala Glu Ala Ala Arg
            20                  25                  30
Ala Pro Ser Val Ser Ala Lys Pro Gly Pro Ala Leu Trp Pro Leu Pro
        35                  40                  45
Leu Leu Val Lys Met Thr Pro Asn Leu Leu His Leu Ala Pro Glu Asn
    50                  55                  60
Phe Tyr Ile Ser His Ser Pro Asn Ser Thr Ala Gly Pro Ser Cys Thr
65                  70                  75                  80
Leu Leu Glu Glu Ala Phe Arg Arg Tyr His Gly Tyr Ile Phe Gly Phe
                85                  90                  95
Tyr Lys Trp His His Glu Pro Ala Glu Phe Gln Ala Lys Thr Gln Val
            100                 105                 110
Gln Gln Leu Leu Val Ser Ile Thr Leu Gln Ser Glu Cys Asp Ala Phe
        115                 120                 125
Pro Asn Ile Ser Ser Asp Glu Ser Tyr Thr Leu Leu Val Lys Glu Pro
    130                 135                 140
Val Ala Val Leu Lys Ala Asn Arg Val Trp Gly Ala Leu Arg Gly Leu
145                 150                 155                 160
Glu Thr Phe Ser Gln Leu Val Tyr Gln Asp Ser Tyr Gly Thr Phe Thr
                165                 170                 175
Ile Asn Glu Ser Thr Ile Ile Asp Ser Pro Arg Phe Ser His Arg Gly
            180                 185                 190
Ile Leu Ile Asp Thr Ser Arg His Tyr Leu Pro Val Lys Ile Ile Leu
```

```
           195                   200                   205
Lys Thr Leu Asp Ala Met Ala Phe Asn Lys Phe Asn Val Leu His Trp
    210                   215                   220
His Ile Val Asp Asp Gln Ser Phe Pro Tyr Gln Ser Ile Thr Phe Pro
225                   230                   235                   240
Glu Leu Ser Asn Lys Gly Ser Tyr Ser Leu Ser His Val Tyr Thr Pro
                245                   250                   255
Asn Asp Val Arg Met Val Ile Glu Tyr Ala Arg Leu Arg Gly Ile Arg
                260                   265                   270
Val Leu Pro Glu Phe Asp Thr Pro Gly His Thr Leu Ser Trp Gly Lys
        275                   280                   285
Gly Gln Lys Asp Leu Leu Thr Pro Cys Tyr Ser Arg Gln Asn Lys Leu
    290                   295                   300
Asp Ser Phe Gly Pro Ile Asn Pro Thr Leu Asn Thr Thr Tyr Ser Phe
305                   310                   315                   320
Leu Thr Thr Phe Phe Lys Glu Ile Ser Glu Val Phe Pro Asp Gln Phe
                325                   330                   335
Ile His Leu Gly Gly Asp Glu Val Glu Phe Lys Cys Trp Glu Ser Asn
                340                   345                   350
Pro Lys Ile Gln Asp Phe Met Arg Gln Lys Gly Phe Gly Thr Asp Phe
        355                   360                   365
Lys Lys Leu Glu Ser Phe Tyr Ile Gln Lys Val Leu Asp Ile Ile Ala
    370                   375                   380
Thr Ile Asn Lys Gly Ser Ile Val Trp Gln Glu Val Phe Asp Asp Lys
385                   390                   395                   400
Ala Lys Leu Ala Pro Gly Thr Ile Val Glu Val Trp Lys Asp Ser Ala
                405                   410                   415
Tyr Pro Glu Glu Leu Ser Arg Val Thr Ala Ser Gly Phe Pro Val Ile
                420                   425                   430
Leu Ser Ala Pro Trp Tyr Leu Asp Leu Ile Ser Tyr Gly Gln Asp Trp
        435                   440                   445
Arg Lys Tyr Tyr Lys Val Glu Pro Leu Asp Phe Gly Gly Thr Gln Lys
    450                   455                   460
Gln Lys Gln Leu Phe Ile Gly Gly Glu Ala Cys Leu Trp Gly Glu Tyr
465                   470                   475                   480
Val Asp Ala Thr Asn Leu Thr Pro Arg Leu Trp Pro Arg Ala Ser Ala
                485                   490                   495
Val Gly Glu Arg Leu Trp Ser Ser Lys Asp Val Arg Asp Met Asp Asp
        500                   505                   510
Ala Tyr Asp Arg Leu Thr Arg His Arg Cys Arg Met Val Glu Arg Gly
        515                   520                   525
Ile Ala Ala Gln Pro Leu Tyr Ala Gly Tyr Cys Asn His Glu Asn Met
    530                   535                   540
```

<210> 13
<211> 529
<212> PRT.
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 13
```
Met Thr Ser Ser Arg Leu Trp Phe Ser Leu Leu Leu Ala Ala Ala Phe
  1               5                  10                  15
Ala Gly Arg Ala Thr Ala Leu Trp Pro Trp Pro Gln Asn Phe Gln Thr
                20                  25                  30
Ser Asp Gln Arg Tyr Val Leu Tyr Pro Asn Asn Phe Gln Phe Gln Tyr
```

```
              35                          40                          45
Asp Val Ser Ser Ala Ala Gln Pro Gly Cys Ser Val Leu Asp Glu Ala
    50                          55                          60
Phe Gln Arg Tyr Arg Asp Leu Leu Phe Gly Ser Gly Ser Trp Pro Arg
65                          70                          75                          80
Pro Tyr Leu Thr Gly Lys Arg His Thr Leu Glu Lys Asn Val Leu Val
                85                          90                          95
Val Ser Val Val Thr Pro Gly Cys Asn Gln Leu Pro Thr Leu Glu Ser
            100                         105                         110
Val Glu Asn Tyr Thr Leu Thr Ile Asn Asp Asp Gln Cys Leu Leu Leu
        115                         120                         125
Ser Glu Thr Val Trp Gly Ala Leu Arg Gly Leu Glu Thr Phe Ser Gln
    130                         135                         140
Leu Val Trp Lys Ser Ala Glu Gly Thr Phe Phe Ile Asn Lys Thr Glu
145                         150                         155                         160
Ile Glu Asp Phe Pro Arg Phe Pro His Arg Gly Leu Leu Leu Asp Thr
                165                         170                         175
Ser Arg His Tyr Leu Pro Leu Ser Ser Ile Leu Asp Thr Leu Asp Val
                180                         185                         190
Met Ala Tyr Asn Lys Leu Asn Val Phe His Trp His Leu Val Asp Asp
            195                         200                         205
Pro Ser Phe Pro Tyr Glu Ser Phe Thr Phe Pro Glu Leu Met Arg Lys
    210                         215                         220
Gly Ser Tyr Asn Pro Val Thr His Ile Tyr Thr Ala Gln Asp Val Lys
225                         230                         235                         240
Glu Val Ile Glu Tyr Ala Arg Leu Arg Gly Ile Arg Val Leu Ala Glu
                245                         250                         255
Phe Asp Thr Pro Gly His Thr Leu Ser Trp Gly Pro Gly Ile Pro Gly
            260                         265                         270
Leu Leu Thr Pro Cys Tyr Ser Gly Ser Glu Pro Ser Gly Thr Phe Gly
        275                         280                         285
Pro Val Asn Pro Ser Leu Asn Asn Thr Tyr Glu Phe Met Ser Thr Phe
    290                         295                         300
Phe Leu Glu Val Ser Ser Val Phe Pro Asp Phe Tyr Leu His Leu Gly
305                         310                         315                         320
Gly Asp Glu Val Asp Phe Thr Cys Trp Lys Ser Asn Pro Glu Ile Gln
            325                         330                         335
Asp Phe Met Arg Lys Lys Gly Phe Gly Glu Asp Phe Lys Gln Leu Glu
        340                         345                         350
Ser Phe Tyr Ile Gln Thr Leu Leu Asp Ile Val Ser Ser Tyr Gly Lys
    355                         360                         365
Gly Tyr Val Val Trp Gln Glu Val Phe Asp Asn Lys Val Lys Ile Gln
    370                         375                         380
Pro Asp Thr Ile Ile Gln Val Trp Arg Glu Asp Ile Pro Val Asn Tyr
385                         390                         395                         400
Met Lys Glu Leu Glu Leu Val Thr Lys Ala Gly Phe Arg Ala Leu Leu
                405                         410                         415
Ser Ala Pro Trp Tyr Leu Asn Arg Ile Ser Tyr Gly Pro Asp Trp Lys
            420                         425                         430
Asp Phe Tyr Val Val Glu Pro Leu Ala Phe Glu Gly Thr Pro Glu Gln
        435                         440                         445
Lys Ala Leu Val Ile Gly Gly Glu Ala Cys Met Trp Gly Glu Tyr Val
    450                         455                         460
Asp Asn Thr Asn Leu Val Pro Arg Leu Trp Pro Arg Ala Gly Ala Val
465                         470                         475                         480
Ala Glu Arg Leu Trp Ser Asn Lys Leu Thr Ser Asp Leu Thr Phe Ala
                485                         490                         495
Tyr Glu Arg Leu Ser His Phe Arg Cys Glu Leu Leu Arg Arg Gly Val
```

```
                500                   505               510
Gln Ala Gln Pro Leu Asn Val Gly Phe Cys Glu Gln Glu Phe Glu Gln
            515               520               525
Thr
```

<210> 14
<211> 739
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

```
<400> 14
ttttaatcct ccgtttttct gcttctgaag ttacttcagc ctggcaagtc ctttacctcc    60
ccgtaggcct ggcgagctgc atcacaacat tcaagattca ccctagagcc atctgggaaa   120
ctttcttctc caggtcgccc tgcgtcctcg cctccccacc ccgttcttct cgagtcgggt   180
gagctgtcta gttccatcac ggccggcacg gccgcagggg tggccggtta tttactgctc   240
tactgggccc gtgagcagtc tggcgagccg agcagttgcc gacgcccggc acaatccgct   300
gcacgtagca ggagcctcag gtccaggccg gaagtgaaag ggcagggtgt gggtcctcct   360
ggggtcgcag gcgcagagcc gcctctggtc acgtgattcg ccgataagtc acgggggcgc   420
cgctcacctg accagggtct cacgtggcca gcccctccg agaggggaga ccagcgggcc   480
atgacaagct ccaggctttg gttttcgctg ctgctggcgg cagcgttcgc aggacgggcg   540
acggccctct ggccctggcc tcagaacttc caaacctccg accagcgcta cgtcctttac   600
ccgaacaact ttcaattcca gtacgatgtc agctcggccg cgcagcccgg ctgctcagtc   660
ctcgacgagg ccttccagcg ctatcgtgac ctgcttttcg gttccgggtc ttggccccgt   720
ccttacctca caggtgagt                                               739
```

<210> 15
<211> 556
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

```
<400> 15
Met Glu Leu Cys Gly Leu Gly Leu Pro Arg Pro Pro Met Leu Leu Ala
1               5               10              15
Leu Leu Leu Ala Thr Leu Leu Ala Ala Met Leu Ala Leu Leu Thr Gln
            20              25              30
Val Ala Leu Val Val Gln Val Ala Glu Ala Ala Arg Ala Pro Ser Val
            35              40              45
Ser Ala Lys Pro Gly Pro Ala Leu Trp Pro Leu Pro Leu Ser Val Lys
        50              55              60
Met Thr Pro Asn Leu Leu His Leu Ala Pro Glu Asn Phe Tyr Ile Ser
65              70              75              80
His Ser Pro Asn Ser Thr Ala Gly Pro Ser Cys Thr Leu Leu Glu Glu
                85              90              95
Ala Phe Arg Arg Tyr His Gly Tyr Ile Phe Gly Phe Tyr Lys Trp His
            100             105             110
His Glu Pro Ala Glu Phe Gln Ala Lys Thr Gln Val Gln Gln Leu Leu
        115             120             125
Val Ser Ile Thr Leu Gln Ser Glu Cys Asp Ala Phe Pro Asn Ile Ser
        130             135             140
Ser Asp Glu Ser Tyr Thr Leu Leu Val Lys Glu Pro Val Ala Val Leu
```

```
145                      150                   155                   160
Lys Ala Asn Arg Val Trp Gly Ala Leu Arg Gly Leu Glu Thr Phe Ser
                    165              170              175
Gln Leu Val Tyr Gln Asp Ser Tyr Gly Thr Phe Thr Ile Asn Glu Ser
                180              185              190

Thr Ile Ile Asp Ser Pro Arg Phe Ser His Arg Gly Ile Leu Ile Asp
            195              200              205
Thr Ser Arg His Tyr Leu Pro Val Lys Ile Ile Leu Lys Thr Leu Asp
        210              215              220
Ala Met Ala Phe Asn Lys Phe Asn Val Leu His Trp His Ile Val Asp
225              230              235              240
Asp Gln Ser Phe Pro Tyr Gln Ser Ile Thr Phe Pro Glu Leu Ser Asn
                245              250              255
Lys Gly Ser Tyr Ser Leu Ser His Val Tyr Thr Pro Asn Asp Val Arg
                260              265              270
Met Val Ile Glu Tyr Ala Arg Leu Arg Gly Ile Arg Val Leu Pro Glu
            275              280              285
Phe Asp Thr Pro Gly His Thr Leu Ser Trp Gly Lys Gly Gln Lys Asp
    290              295              300
Leu Leu Thr Pro Cys Tyr Ser Arg Gln Asn Lys Leu Asp Ser Phe Gly
305              310              315              320
Pro Ile Asn Pro Thr Leu Asn Thr Thr Tyr Ser Phe Leu Thr Thr Phe
                325              330              335
Phe Lys Glu Ile Ser Glu Val Phe Pro Asp Gln Phe Ile His Leu Gly
            340              345              350
Gly Asp Glu Val Glu Phe Lys Cys Trp Glu Ser Asn Pro Lys Ile Gln
        355              360              365
Asp Phe Met Arg Gln Lys Gly Phe Gly Thr Asp Phe Lys Lys Leu Glu
    370              375              380
Ser Phe Tyr Ile Gln Lys Val Leu Asp Ile Ile Ala Thr Ile Asn Lys
385              390              395              400
Gly Ser Ile Val Trp Gln Glu Val Phe Asp Asp Lys Ala Lys Leu Ala
            405              410              415
Pro Gly Thr Ile Val Glu Val Trp Lys Asp Ser Ala Tyr Pro Glu Glu
            420              425              430
Leu Ser Arg Val Thr Ala Ser Gly Phe Pro Val Ile Leu Ser Ala Pro
        435              440              445
Trp Tyr Leu Asp Leu Ile Ser Tyr Gly Gln Asp Trp Arg Lys Tyr Tyr
    450              455              460
Lys Val Glu Pro Leu Asp Phe Gly Gly Thr Gln Lys Gln Lys Gln Leu
465              470              475              480
Phe Ile Gly Gly Glu Ala Cys Leu Trp Gly Glu Tyr Val Asp Ala Thr
            485              490              495
Asn Leu Thr Pro Arg Leu Trp Pro Arg Ala Ser Ala Val Gly Glu Arg
        500              505              510
Leu Trp Ser Ser Lys Asp Val Arg Asp Met Asp Asp Ala Tyr Asp Arg
        515              520              525
Leu Thr Arg His Arg Cys Arg Met Val Glu Arg Gly Ile Ala Ala Gln
    530              535              540
Pro Leu Tyr Ala Gly Tyr Cys Asn His Glu Asn Met
545              550              555
```

<210> 16
<211> 1857
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 16
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ctgatccggg ccgggcggga agtcgggtcc cgaggctccg gctcggcaga ccgggcggaa   60
agcagccgag cggccatgga gctgtgcggg ctggggctgc cccggccgcc catgctgctg  120
gcgctgctgt tggcgacact gctggcggcg atgttggcgc tgctgactca ggtggcgctg  180
gtggtgcagg tggcggaggc ggctcgggcc ccgagcgtct cggccaagcc ggggccggcg  240
ctgtggcccc tgccgctctc ggtgaagatg accccgaacc tgctgcatct cgccccggag  300
aacttctaca tcagccacag ccccaattcc acggcgggcc cctcctgcac cctgctggag  360
gaagcgtttc gacgatatca tggctatatt tttggtttct acaagtggca tcatgaacct  420
gctgaattcc aggctaaaac ccaggttcag caacttcttg tctcaatcac ccttcagtca  480
gagtgtgatg ctttccccaa catatcttca gatgagtctt atactttact tgtgaaagaa  540
ccagtggctg tccttaaggc caacagagtt tggggagcat tacgaggttt agagaccttt  600
agccagttag tttatcaaga ttcttatgga actttcacca tcaatgaatc caccattatt  660
gattctccaa ggttttctca cagaggaatt ttgattgata catccagaca ttatctgcca  720
gttaagatta ttcttaaaac tctggatgcc atggctttta ataagtttaa tgttcttcac  780
tggcacatag ttgatgacca gtctttccca tatcagagca tcactttttcc tgagttaagc  840
aataaaggaa gctattcttt gtctcatgtt tatacaccaa atgatgtccg tatggtgatt  900
gaatatgcca gattacgagg aattcgagtc ctgccagaat ttgataccc tgggcataca  960
ctatcttggg gaaaaggtca gaaagacctc ctgactccat gttacagtag acaaaacaag 1020
ttggactctt ttggacctat aaaccctact ctgaatacaa catacagctt ccttactaca 1080
tttttcaaag aaattagtga ggtgtttcca gatcaattca ttcatttggg aggagatgaa 1140
gtggaattta aatgttggga atcaaatcca aaaattcaag atttcatgag gcaaaaaggc 1200
tttggcacag attttaagaa actagaatct ttctacattc aaaaggtttt ggatattatt 1260
gcaaccataa acaagggatc cattgtctgg caggaggttt ttgatgataa agcaaagctt 1320
gcgccgggca caatagttga agtatggaaa gacagcgcat atcctgagga actcagtaga 1380
gtcacagcat ctggcttccc tgtaatcctt tctgctcctt ggtacttaga tttgattagc 1440
tatggacaag attggaggaa atactataaa gtggaacctc ttgattttgg cggtactcag 1500
aaacagaaac aacttttcat tggtggagaa gcttgtctat ggggagaata tgtggatgca 1560
actaacctca ctccaagatt atggcctcgg gcaagtgctg ttggtgagag actctggagt 1620
tccaaagatg tcagagatat ggatgacgcc tatgacagac tgacaaggca ccgctgcagg 1680
atggtcgaac gtggaatagc tgcacaacct ctttatgctg gatattgtaa ccatgagaac 1740
atgtaaaaaa tggaggggaa aaaggccaca gcaatctgta ctacaatcaa ctttattttg 1800
aaatcatgta aataagata ttagactttt ttgaataaaa tatttttatt gattgaa     1857
```

<210> 17
<211> 536
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 17
```
Met Pro Gln Ser Pro Arg Ser Ala Pro Gly Leu Leu Leu Leu Gln Ala
1               5                   10                  15
Leu Val Ser Leu Val Ser Leu Ala Leu Val Ala Pro Ala Arg Leu Gln
            20                  25                  30
Pro Ala Leu Trp Pro Phe Pro Arg Ser Val Gln Met Phe Pro Arg Leu
        35                  40                  45
Leu Tyr Ile Ser Ala Glu Asp Phe Ser Ile Asp His Ser Pro Asn Ser
    50                  55                  60
Thr Ala Gly Pro Ser Cys Ser Leu Leu Gln Glu Ala Phe Arg Arg Tyr
65                  70                  75                  80
Tyr Asn Tyr Val Phe Gly Phe Tyr Lys Arg His His Gly Pro Ala Arg
                85                  90                  95
Phe Arg Ala Glu Pro Gln Leu Gln Lys Leu Leu Val Ser Ile Thr Leu
```

```
             100                    105                    110
Glu Ser Glu Cys Glu Ser Phe Pro Ser Leu Ser Ser Asp Glu Thr Tyr
        115                    120                    125
Ser Leu Leu Val Gln Glu Pro Val Ala Val Leu Lys Ala Asn Ser Val
    130                    135                    140

Trp Gly Ala Leu Arg Gly Leu Glu Thr Phe Ser Gln Leu Val Tyr Gln
145                 150                    155                    160
Asp Ser Phe Gly Thr Phe Thr Ile Asn Glu Ser Ser Ile Ala Asp Ser
                165                    170                    175
Pro Arg Phe Pro His Arg Gly Ile Leu Ile Asp Thr Ser Arg His Phe
            180                    185                    190
Leu Pro Val Lys Thr Ile Leu Lys Thr Leu Asp Ala Met Ala Phe Asn
        195                    200                    205
Lys Phe Asn Val Leu His Trp His Ile Val Asp Asp Gln Ser Phe Pro
    210                    215                    220
Tyr Gln Ser Thr Thr Phe Pro Glu Leu Ser Asn Lys Gly Ser Tyr Ser
225                 230                    235                    240
Leu Ser His Val Tyr Thr Pro Asn Asp Val Arg Met Val Leu Glu Tyr
                245                    250                    255
Ala Arg Leu Arg Gly Ile Arg Val Ile Pro Glu Phe Asp Thr Pro Gly
            260                    265                    270
His Thr Gln Ser Trp Gly Lys Gly Gln Lys Asn Leu Leu Thr Pro Cys
        275                    280                    285
Tyr Asn Gln Lys Thr Lys Thr Gln Val Phe Gly Pro Val Asp Pro Thr
    290                    295                    300
Val Asn Thr Thr Tyr Ala Phe Phe Asn Thr Phe Phe Lys Glu Ile Ser
305                 310                    315                    320
Ser Val Phe Pro Asp Gln Phe Ile His Leu Gly Gly Asp Glu Val Glu
                325                    330                    335
Phe Gln Cys Trp Ala Ser Asn Pro Asn Ile Gln Gly Phe Met Lys Arg
            340                    345                    350
Lys Gly Phe Gly Ser Asp Phe Arg Arg Leu Glu Ser Phe Tyr Ile Lys
        355                    360                    365
Lys Ile Leu Glu Ile Ile Ser Ser Leu Lys Lys Asn Ser Ile Val Trp
    370                    375                    380
Gln Glu Val Phe Asp Asp Lys Val Glu Leu Gln Pro Gly Thr Val Val
385                 390                    395                    400
Glu Val Trp Lys Ser Glu His Tyr Ser Tyr Glu Leu Lys Gln Val Thr
                405                    410                    415
Gly Ser Gly Phe Pro Ala Ile Leu Ser Ala Pro Trp Tyr Leu Asp Leu
            420                    425                    430
Ile Ser Tyr Gly Gln Asp Trp Lys Asn Tyr Tyr Lys Val Glu Pro Leu
        435                    440                    445
Asn Phe Glu Gly Ser Glu Lys Gln Lys Gln Leu Val Ile Gly Gly Glu
    450                    455                    460
Ala Cys Leu Trp Gly Glu Phe Val Asp Ala Thr Asn Leu Thr Pro Arg
465                 470                    475                    480
Leu Trp Pro Arg Ala Ser Ala Val Gly Glu Arg Leu Trp Ser Pro Lys
                485                    490                    495
Thr Val Thr Asp Leu Glu Asn Ala Tyr Lys Arg Leu Ala Val His Arg
            500                    505                    510
Cys Arg Met Val Ser Arg Gly Ile Ala Ala Gln Pro Leu Tyr Thr Gly
        515                    520                    525
Tyr Cys Asn Tyr Glu Asn Lys Ile
    530                    535
```

<210> 18
<211> 1750

```
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 18
ggagcagtca tgccgcagtc cccgcgtagc gcccccgggc tgctgctgct gcaggcgctg      60
gtgtcgctag tgtcgctggc cctagtggcc ccggcccgac tgcaacctgc gctatggccc     120
ttcccgcgct cggtgcagat gttcccgcgg ctgttgtaca tctccgcgga ggacttcagc     180
atcgaccaca gtcccaattc cacagcgggc ccttcctgct cgctgctaca ggaggcgttt     240
cggcgatatt acaactatgt ttttggtttc tacaagagac atcatggccc tgctagattt     300
cgagctgagc cacagttgca gaagctcctg gtctccatta ccctcgagtc agagtgcgag     360
tccttcccta gtctgtcttc agatgaaacc tattctctgc ttgtacaaga accagtagcc     420
gtcctcaagg ccaacagcgt ttggggagcg ttacgaggtt tagagacgtt tagccagtta     480
gtttaccaag actctttcgg gactttcacc atcaatgaat ccagtatagc tgattctcca     540
agattccctc atagaggaat tttaattgat acatctagac acttcctgcc tgtgaagaca     600
attttaaaaa ctctggatgc catggctttt aataagttta atgttcttca ctggcacata     660
gtggacgacc agtctttccc ttatcagagt accactttttc ctgagctaag caataaggga     720
agctactctt tgtctcatgt ctatacacca aacgatgtcc ggatggtgct ggagtacgcc     780
cggctccgag ggattcgagt cataccagaa tttgataccc ctggccatac acagtcttgg     840
ggcaaaggac agaaaaacct tctaactcca tgttacaatc aaaaaaactaa aactcaagtg     900
tttgggcctg tagacccaac tgtaaacaca acgtatgcat tctttaacac attttttcaaa     960
gaaatcagca gtgtgtttcc agatcagttc atccacttgg gaggagatga agtagaattt    1020
caatgttggg catcaaatcc aaacatccaa ggtttcatga agagaaaggg ctttggcagc    1080
gattttagaa gactagaatc cttttatatt aaaaagattt tggaaattat ttcatcctta    1140
aagaagaact ccattgtttg gcaagaagtt tttgatgata aggtggagct tcagccgggc    1200
acagtagtcg aagtgtggaa gagtgagcat tattcatatg agctaaagca agtcacaggc    1260
tctggcttcc ctgccatcct ttctgctcct tggtacttag acctgatcag ctatgggcaa    1320
gactggaaaa actactacaa agttgagccc cttaattttg aaggctctga gaagcagaaa    1380
caacttgtta ttggtggaga agcttgcctg tggggagaat ttgtggatgc aactaacctt    1440
actccaagat tatggcctcg agcaagcgct gttggtgaga gactctggag ccctaaaact    1500
gtcactgacc tagaaaatgc ctacaaacga ctggccgtgc accgctgcag aatggtcagc    1560
cgtggaatag ctgcacaacc tctctatact ggatactgta actatgagaa taaaatatag    1620
aagtgacaga cgtctacagc attccagcta tgatcatgtt gattctgaaa tcatgtaaat    1680
taagatttgt taggctgttt tttttttaaa taaaccatct ttttattgat tgaatctttc    1740
taaaaaaaaa                                                          1750

<210> 19
<211> 12263
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 19
aatgtagtct tatgcaatac tcttgtagtc ttgcaacatg gtaacgatga gttagcaaca      60
tgccttacaa ggagagaaaa agcaccgtgc atgccgattg gtggaagtaa ggtggtacga     120
tcgtgcctta ttaggaaggc aacagacggg tctgacatgg attggacgaa ccactgaatt     180
gccgcattgc agagatattg tatttaagtg cctagctcga tacataaacg ggtctctctg     240
gttagaccag atctgagcct gggagctctc tggctaacta gggaacccac tgcttaagcc     300
tcaataaagc ttgccttgag tgcttcaagt agtgtgtgcc cgtctgttgt gtgactctgg     360
taactagaga tccctcagac ccttttagtc agtgtggaaa atctctagca gtggcgcccg     420
aacagggact tgaaagcgaa agggaaacca gaggagctct ctcgacgcag gactcggctt     480
gctgaagcgc gcacggcaag aggcgagggg cggcgactgg tgagtacgcc aaaaattttg     540
actagcggag gctagaagga gagagatggg tgcgagagcg tcagtattaa gcggggga
ga     600
```

```
attagatcgc gatgggaaaa aattcggtta aggccagggg gaaagaaaaa atataaatta    660
aaacatatag tatgggcaag cagggagcta gaacgattcg cagttaatcc tggcctgtta    720
gaaacatcag aaggctgtag acaaatactg ggacagctac aaccatccct tcagacagga    780
tcagaagaac ttagatcatt atataataca gtagcaaccc tctattgtgt gcatcaaagg    840
atagagataa aagacaccaa ggaagcttta gacaagatag aggaagagca aaacaaaagt    900
aagaccaccg cacagcaagc ggccgctgat cttcagacct ggaggaggag atatgaggga    960
caattggaga agtgaattat ataaatataa agtagtaaaa attgaaccat taggagtagc   1020
acccaccaag gcaaagagaa gagtggtgca gagagaaaaa agagcagtgg gaataggagc   1080
tttgttcctt gggttcttgg gagcagcagg aagcactatg ggcgcagcgt caatgacgct   1140
gacggtacag gccagacaat tattgtctgg tatagtgcag cagcagaaca atttgctgag   1200
ggctattgag gcgcaacagc atctgttgca actcacagtc tggggcatca agcagctcca   1260
ggcaagaatc ctggctgtgg aaagatacct aaaggatcaa cagctcctgg ggatttgggg   1320
ttgctctgga aaactcattt gcaccactgc tgtgccttgg aatgctagtt ggagtaataa   1380
atctctggaa cagatttgga atcacacgac ctggatggag tgggacagag aaattaacaa   1440
ttacacaagc ttaatacact ccttaattga agaatcgcaa aaccagcaag aaaagaatga   1500
acaagaatta ttggaattag ataaatgggc aagtttgtgg aattggttta acataacaaa   1560
ttggctgtgg tatataaaat tattcataat gatagtagga ggcttggtag gtttaagaat   1620
agttttttgct gtactttcta tagtgaatag agttaggcag ggatattcac cattatcgtt   1680
tcagacccac ctcccaaccc cgaggggacc cgacaggccc gaaggaatag aagaagaagg   1740
tggagagaga gacagagaca gatccattcg attagtgaac ggatctcgac ggtatcgata   1800
agcttgatat cgaattcggt accctagtta ttaatagtaa tcaattacgg ggtcattagt   1860
tcatagccca tatatggagt tccgcgttac ataacttacg gtaaatggcc cgcctggctg   1920
accgcccaac gacccccgcc cattgacgtc aataatgacg tatgttccca tagtaacgcc   1980
aataggggact ttccattgac gtcaatgggt ggactattta cggtaaactg cccacttggc   2040
agtacatcaa gtgtatcata tgccaagtac gccccctatt gacgtcaatg acggtaaatg   2100
gcccgcctgg cattatgccc agtacatgac cttatgggac tttcctactt ggcagtacat   2160
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ggggcggggc ggggcgaggc ggagaggtgc ggcggcagcc aatcagagcg gcgcgctccg   2400
aaagtttcct tttatggcga ggcggcggcg gcggccgccc tataaaaagc gaagcgcgcg   2460
gcgggcggga gtcgctgcga cgctgccttc gccccgtgcc ccgctccgcc gccgcctcgc   2520
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acggggcgtg gcgcggggct cgccgtgccg ggcggggggt ggcggcaggt gggggtgccg   3060
ggcggggcgg ggccgcctcg ggccggggag ggctcggggg aggggcgcgg cggcccccgg   3120
agcgccggcg gctgtcgagg cgcggcgagc cgcagccatt gccttttatg gtaatcgtgc   3180
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cccctcctgc accctgctgg aggaagcgtt tcgacgtatat catggctata ttttttggttt   4020
ctacaagtgg catcatgaac ctgctgaatt ccaggctaaa acccaggttc agcaacttct   4080
tgtctcaatc acccttcagt cagagtgtga tgctttcccc aacatatctt cagatgagtc   4140
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agatttcatg aggcaaaaag gctttggcac agattttaag aaactagaat ctttctacat    4860
tcaaaaggtt ttggatatta ttgcaaccat aaacaaggga tccattgtct ggcaggaggt    4920
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atatcctgag gaactcagta gagtcacagc atctggcttc cctgtaatcc tttctgctcc    5040
ttggtactta gatttgatta gctatggaca agattggagg aaatactata aagtggaacc    5100
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tgttggtgag agactctgga gttccaaaga tgtcagagat atggatgacg cctatgacag    5280
actgacaagg caccgctgca ggatggtcga acgtggaata gctgcacaac ctctttatgc    5340
tggatattgt aaccatgaga acatgtaaaa aatggagggg aaaaaggcca cagcaatctg    5400
tactacaatc aactttattt tgaaatcatg taaaataaga tattagactt ttttgaataa    5460
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gcgacccttt gcaggcagcg gaaccccca cctggcgaca ggtgcctctg cggccaaaag    5820
ccacgtgtat aagatacacc tgcaaaggcg gcacaacccc agtgccacgt tgtgagttgg    5880
atagttgtgg aaagagtcaa atggctctcc tcaagcgtat tcaacaaggg gctgaaggat    5940
gcccagaagg tacccattg tatgggatct gatctggggc ctcggtgcac atgctttaca    6000
tgtgtttagt cgaggttaaa aaaacgtcta ggccccccga accacgggga cgtggttttc    6060
ctttgaaaaa cacgatgata agcttgccac aacccgggat cctctatgac aagctccagg    6120
ctttggtttt cgctgctgct ggcggcagcg ttcgcaggac gggcgacggc cctctggccc    6180
tggcctcaga acttccaaac ctccgaccag cgctacgtcc tttacccgaa caactttcaa    6240
ttccagtacg atgtcagctc ggccgcgcag cccggctgct cagtcctcga cgaggccttc    6300
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aaacggcata cactggagaa gaatgtgttg gttgtctctg tagtcacacc tggatgtaac    6420
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ttactcctct ctgagactgt ctggggagct ctccgaggtc tggagacttt tagccagctt    6540
gtttggaaat ctgctgaggg cacattcttt atcaacaaga ctgagattga ggactttccc    6600
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gtacctttaa gaccaatgac ttacaaggca gctgtagatc ttagccactt tttaaaagaa   7620
aaggggggac tggaagggct aattcactcc caacgaagac aagatctgct ttttgcttgt   7680
actgggtctc tctggttaga ccagatctga gcctgggagc tctctggcta actagggaac   7740
ccactgctta agcctcaata aagcttgcct tgagtgcttc aagtagtgtg tgcccgtctg   7800
ttgtgtgact ctggtaacta gagatccctc agacccttt agtcagtgtg aaaatctct   7860
agcagtagta gttcatgtca tcttattatt cagtatttat aacttgcaaa gaaatgaata   7920
tcagagagtg agaggaactt gtttattgca gcttataatg gttacaaata aagcaatagc   7980
atcacaaatt tcacaaataa agcattttt tcactgcatt ctagttgtgg tttgtccaaa   8040
ctcatcaatg tatcttatca tgtctggctc tagctatccc gccccaact ccgcccatcc   8100
cgccctaac tccgcccagt tccgcccatt ctcgcccca tggctgacta atttttttta   8160
tttatgcaga ggccgaggcc gcctcggcct ctgagctatt ccagaagtag tgaggaggct   8220
tttttggagg cctaggacg tacccaattc gccctatagt gagtcgtatt acgcgcgctc   8280
actggccgtc gttttacaac gtcgtgactg ggaaaaccct ggcgttaccc aacttaatcg   8340
ccttgcagca catccccctt cgccagctg gcgtaatagc gaagaggccc gcaccgatcg   8400
cccttcccaa cagttgcgca gcctgaatgg cgaatgggac gcgccctgta gcggcgcatt   8460
aagcgcggcg ggtgtggtgg ttacgcgcag cgtgaccgct acacttgcca gcgccctagc   8520
gcccgctcct ttcgctttct tcccttcctt tctcgccacg ttcgccggct ttccccgtca   8580
agctctaaat cggggggctcc ctttagggtt ccgatttagt gctttacggc acctcgaccc   8640
caaaaaactt gattagggtg atggttcacg tagtgggcca tcgccctgat agacggtttt   8700
```

```
tcgcccttttg acgttggagt ccacgttctt taatagtgga ctcttgttcc aaactggaac    8760
aacactcaac cctatctcgg tctattcttt tgatttataa gggattttgc cgatttcggc    8820
ctattggtta aaaaatgagc tgatttaaca aaaatttaac gcgaattta acaaaatatt     8880
aacgcttaca atttaggtgg cacttttcgg ggaaatgtgc gcggaacccc tatttgttta    8940
tttttctaaa tacattcaaa tatgtatccg ctcatgagac aataaccctg ataaatgctt    9000
caataatatt gaaaaaggaa gagtatgagt attcaacatt tccgtgtcgc ccttattccc    9060
ttttttgcgg cattttgcct tcctgttttt gctcacccag aaacgctggt gaaagtaaaa    9120
gatgctgaag atcagttggg tgcacgagtg ggttacatcg aactggatct caacagcggt    9180
aagatccttg agagttttcg ccccgaagaa cgttttccaa tgatgagcac ttttaaagtt    9240
ctgctatgtg gcgcggtatt atcccgtatt gacgccgggc aagagcaact cggtcgccgc    9300
atacactatt ctcagaatga cttggttgag tactcaccag tcacagaaaa gcatcttacg    9360
gatggcatga cagtaagaga attatgcagt gctgccataa ccatgagtga taacactgcg    9420
gccaacttac ttctgacaac gatcggagga ccgaaggagc taaccgcttt tttgcacaac    9480
atgggggatc atgtaactcg ccttgatcgt tgggaaccgg agctgaatga agccatacca    9540
aacgacgagc gtgacaccac gatgcctgta gcaatggcaa caacgttgcg caaactatta    9600
actggcgaac tacttactct agcttcccgg caacaattaa tagactggat ggaggcggat    9660
aaagttgcag gaccacttct gcgctcggcc cttccggctg ctggtttat tgctgataaa     9720
tctggagccg gtgagcgtgg gtctcgcggt atcattgcag cactggggcc agatggtaag    9780
ccctcccgta tcgtagttat ctacacgacg gggagtcagg caactatgga tgaacgaaat    9840
agacagatcg ctgagatagg tgcctcactg attaagcatt ggtaactgtc agaccaagtt    9900
tactcatata tactttagat tgatttaaaa cttcattttt aatttaaaag gatctaggtg    9960
aagatccttt ttgataatct catgaccaaa atcccttaac gtgagttttc gttccactga   10020
gcgtcagacc ccgtagaaaa gatcaaagga tcttcttgag atcctttttt tctgcgcgta   10080
atctgctgct tgcaaacaaa aaaaccaccg ctaccagcgg tggtttgttt gccggatcaa   10140
gagctaccaa ctcttttttcc gaaggtaact ggcttcagca gagcgcagat accaaatact   10200
gttcttctag tgtagccgta gttaggccac cacttcaaga actctgtagc accgcctaca   10260
tacctcgctc tgctaatcct gttaccagtg gctgctgcca gtggcgataa gtcgtgtctt   10320
accgggttgg actcaagacg atagttaccg gataaggcgc agcggtcggg ctgaacgggg   10380
ggttcgtgca cacagcccag cttggagcga acgacctaca ccgaactgag atacctacag   10440
cgtgagctat gagaaagcgc cacgcttccc gaagggagaa aggcggacag gtatccggta   10500
agcggcaggg tcggaacagg agagcgcacg agggagcttc caggggaaa cgcctggtat    10560
ctttatagtc ctgtcgggtt tcgccacctc tgacttgagc gtcgatttt gtgatgctcg    10620
tcaggggggc ggagcctatg gaaaaacgcc agcaacgcgg ccttttacg gttcctggcc    10680
ttttgctggc cttttgctca catgttcttt cctgcgttat cccctgattc tgtggataac   10740
cgtattaccg cctttgagtg agctgatacc gctcgccgca gccgaacgac cgagcgcagc   10800
gagtcagtga gcgaggaagc ggaagagcgc ccaatacgca aaccgcctct ccccgcgcgt   10860
tggccgattc attaatgcag ctggcacgac aggtttcccg actggaaagc gggcagtgag   10920
cgcaacgcaa ttaatgtgag ttagctcact cattaggcac cccaggcttt acactttatg   10980
cttccggctc gtatgttgtg tggaattgtg agcggataac aatttcacac aggaaacagc   11040
tatgaccatg attacgccaa gcgcgcaatt aaccctcact aaagggaaca aaagctggag   11100
ctgcaagctt                                                          11110
```

<210> 21
<211> 1278
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
     Synthetic Construct

<400> 21

```
tcgaggtgag ccccacgttc tgcttcactc tccccatctc cccccctcc ccaccccaa       60
ttttgtattt atttattttt taattatttt gtgcagcgat gggggcgggg ggggggggg    120
cgcgcgccag gcggggcggg gcggggcgag gggcggggcg gggcgaggcg gagaggtgcg    180
gcggcagcca atcagagcgg cgcgctccga aagtttcctt ttatggcgag gcggcggcgg    240
cggcggccct ataaaaagcg aagcgcgcgg cgggcgggag tcgctgcgtt gccttcgccc    300
```

```
cgtgccccgc tccgcgccgc ctcgcgccgc ccgccccggc tctgactgac cgcgttactc    360
ccacaggtga gcgggcggga cggcccttct cctccgggct gtaattagcg cttggtttaa    420
tgacggctcg tttctttct gtggctgcgt gaaagcctta aagggctccg ggagggccct     480
ttgtgcgggg gggagcggct cggggggtgc gtgcgtgtgt gtgtgcgtgg ggagcgccgc    540
gtgcggcccg cgctgcccgg cggctgtgag cgctgcgggc gcggcgcggg gctttgtgcg    600
ctccgcgtgt gcgcgagggg agcgcggccg ggggcggtgc cccgcggtgc ggggggggctg   660
cgagggaac aaaggctgcg tgcggggtgt gtgcgtgggg gggtgagcag ggggtgtggg     720
cgcggcggtc gggctgtaac ccccccctgc accccctcc ccgagttgct gagcacggcc     780
cggcttcggg tgcggggctc cgtgcggggc gtggcgcggg gctcgccgtg ccgggcgggg    840
ggtggcggca ggtgggggtg ccgggcgggg cggggccgcc tcgggccggg gagggctcgg    900
gggaggggcg cggcggcccc ggagcgccgg cggctgtcga ggcgcggcga ccgcagcca     960
ttgccttta tggtaatcgt gcgagagggc gcagggactt cctttgtccc aaatctggcg    1020
gagccgaaat ctgggaggcg ccgccgcacc ccctctagcg ggcgcgggcg aagcggtgcg    1080
gcgccggcag gaaggaaatg ggcggggagg gccttcgtgc gtcgccgcgc cgccgtcccc    1140
ttctccatct ccagcctcgg ggctgccgca gggggacggc tgccttcggg ggggacgggg    1200
cagggcgggg ttcggcttct ggcgtgtgac cggcggggtt tatatcttcc cttctctgtt   1260
cctccgcagc cagccatg                                                 1278
```

<210> 22
<211> 1278
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 22
```
tcgaggtgag ccccacgttc tgcttcactc tccccatctc ccccccctcc ccacccccaa    60
ttttgtattt atttattttt taattatttt gtgcagcgat ggggcggggg ggggggggg    120
cgcgcgccag gcggggcggg gcggggcgag gggcggggcg gggcgaggcg gagaggtgcg   180
gcggcagcca atcagagcgg cgcgctccga aagtttcctt ttatggcgag gcggcggcgg   240
cggcggccct ataaaaagcg aagcgcgcgg cgggcgggag tcgctgcgtt gccttcgccc    300
cgtgccccgc tccgcgccgc ctcgcgccgc ccgccccggc tctgactgac cgcgttactc    360
ccacaggtga gcgggcggga cggcccttct cctccgggct gtaattagcg cttggtttaa    420
tgacggctcg tttctttct gtggctgcgt gaaagcctta aagggctccg ggagggccct     480
ttgtgcgggg gggagcggct cggggggtgc gtgcgtgtgt gtgtgcgtgg ggagcgccgc    540
gtgcggcccg cgctgcccgg cggctgtgag cgctgcgggc gcggcgcggg gctttgtgcg    600
ctccgcgtgt gcgcgagggg agcgcggccg ggggcggtgc cccgcggtgc ggggggggctg   660
cgagggaac aaaggctgcg tgcggggtgt gtgcgtgggg gggtgagcag ggggtgtggg     720
cgcggcggtc gggctgtaac ccccccctgc accccctcc ccgagttgct gcgcacggcc     780
cggcttcggg tgcggggctc cgtgcggggc gtggcgcggg gctcgccgtg ccgggcgggg    840
ggtggcggca ggtgggggtg ccgggcgggg cggggccgcc tcgggccggg gagggctcgg    900
gggaggggcg cggcggcccc ggagcgccgg cggctgtcga ggcgcggcga ccgcagcca     960
ttgccttta tggtaatcgt gcgagagggc gcagggactt cctttgtccc aaatctggcg    1020
gagccgaaat ctgggaggcg ccgccgcacc ccctctagcg ggcgcgggcg aagcggtgcg    1080
gcgccggcag gaaggaaatg ggcggggagg gccttcgtgc gtcgccgcgc cgccgtcccc    1140
ttctccatct ccagcctcgg ggctgccgca gggggacggc tgccttcggg ggggacgggg    1200
cagggcgggg ttcggcttct ggcgttgtac cggcggggtt tatatcttcc cttctctgtt   1260
cctccgcagc cagccatg                                                 1278
```

<210> 23
<211> 1729
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

```
<400> 23
gaattcggta ccctagttat taatagtaat caattacggg gtcattagtt catagcccat      60
atatggagtt ccgcgttaca taacttacgg taaatggccc gcctggctga ccgcccaacg     120
accccgccc attgacgtca ataatgacgt atgttcccat agtaacgcca atagggactt      180
tccattgacg tcaatgggtg gactatttac ggtaaactgc ccacttggca gtacatcaag     240
tgtatcatat gccaagtacg cccccctattg acgtcaatga cggtaaatgg cccgcctggc    300
attatgccca gtacatgacc ttatgggact ttcctacttg gcagtacatc tacgtattag     360
tcatcgctat taccatggtc gaggtgagcc ccacgttctg cttcactctc cccatctccc     420
ccccctcccc accccaatt ttgtatttat ttatttttta attattttgt gcagcgatgg       480
gggcggggggg ggggggggg cgcgcgccag gcggggcggg gcggggcgag gggcggggcg     540
gggcgaggcg gagaggtgcg gcggcagcca atcagagcgg cgcgctccga aagtttcctt     600
ttatggcgag gcggcggcgg cggcggccct ataaaaagcg aagcgcgcgg cgggcgggag     660
tcgctgcgac gctgccttcg ccccgtgccc cgctccgccg ccgcctcgcg ccgcccgccc     720
cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctccg     780
ggctgtaatt agcgcttggt ttaatgacgg cttgtttctt ttctgtggct gcgtgaaagc .    840
cttgagggc tccgggaggg ccctttgtgc gggggggagc ggctcggggg gtgcgtgcgt       900
gtgtgtgtgc gtggggagcg ccgcgtgcgg cccgcgctgc ccggcggctg tgagcgctgc      960
gggcgcggcg cggggctttg tgcgctccgc agtgtgcgcg aggggagcgc ggccggggc     1020
ggtgccccgc ggtgcggggg gggctgcgag gggaacaaag gctgcgtgcg gggtgtgtgc     1080
gtggggggggt gagcagggggg tgtgggcgcg gcggtcgggc tgtaaccccc ccctgcaccc     1140
ccctccccga gttgctgagc acggcccggc ttcgggtgcg gggctccgta cggggcgtgg     1200
cgcggggctc gccgtgccgg gcggggggtg gcggcaggtg ggggtgccgg gcggggcggg     1260
gccgcctcgg gccggggagg gctcggggga ggggcgcggc ggccccggga gcgccggcgg     1320
ctgtcgaggc gcggcgagcc gcagccattg ccttttatgg taatcgtgcg agagggcgca     1380
gggacttcct ttgtcccaaa tctgtgcgga gccgaaatct gggaggcgcc gccgcacccc     1440
ctctagcggg cgcggggcga agcggtgcgg cgccggcagg aaggaaatgg gcggggaggg     1500
ccttcgtgcg tcgccgcgcc gccgtcccct tctccctctc cagcctcggg gctgtccgcg     1560
gggggacggc tgccttcggg ggggacgggg cagggcgggg ttcggcttct ggcgtgtgac     1620
cggcggctct agagcctctg ctaaccatgt tcatgccttc ttctttttcc tacagctcct     1680
gggcaacgtg ctggttattg tgctgtctca tcattttggc aaagaattc                 1729
```

```
<210> 24
<211> 366
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct
```

```
<400> 24
tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata tggagttccg      60
cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt     120
gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca     180
atgggtggac tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc     240
aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta     300
catgaccta tgggactttc ctacttggca gtacatctac gtattagtca tcgctattac     360
catggt                                                               366
```

```
<210> 25
<211> 1295
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct
```

```
<400> 25
ccaattttgt atttattat ttttaatta ttttgtgcag cgatggggc ggggggggg      60
ggggggcgcg cgccaggcgg ggcggggcgg ggcgagggc gggcgggc gaggcggaga     120
ggtgcggcgg cagccaatca gagcggcgcg ctccgaaagt ttccttttat ggcgaggcgg   180
cggcggcggc ggccctataa aaagcgaagc gcgcggcggg cgggagtcgc tgcgacgctg   240
ccttcgcccc gtgccccgct ccgccgccgc ctcgcgccgc ccgccccggc tctgactgac   300
cgcgttactc ccacaggtga gcgggcggga cggcccttct cctccgggct gtaattagcg   360
cttggtttaa tgacggcttg tttcttttct gtggctgcgt gaaagccttg aggggctccg   420
ggagggccct ttgtgcgggg gggagcggct cggggggtgc gtgcgtgtgt gtgtgcgtgg   480
ggagcgccgc gtgcggcccg cgctgcccgg cggctgtgag cgctgcgggc gcggcgcggg   540
gctttgtgcg ctccgcagtg tgcgcgaggg gagcgcggcc ggggcggtg ccccgcggtg    600
cggggggggc tgcgagggga acaaaggctg cgtgcggggt gtgtgcgtgg ggggtgagc    660
aggggtgtg ggcgcggcgg tcgggctgta acccccccct gcaccccccct ccccgagttg   720
ctgagcacgg cccggcttcg ggtgcggggc tccgtacggg gcgtggcgcg gggctcgccg   780
tgccgggcgg ggggtggcgg caggtggggg tgccgggcgg ggcggggccg cctcgggccg   840
gggagggctc gggggagggg cgcggcggcc cccggagcgc cggcggctgt cgaggcgcgg   900
cgagccgcag ccattgcctt ttatggtaat cgtgcgagag ggcgcaggga cttcctttgt   960
cccaaatctg tgcggagccg aaatctggga ggcgccgccg cacccccctct agcgggcgcg  1020
gggcgaagcg gtgcggcgcc ggcaggaagg aaatgggcgg ggagggcctt cgtgcgtcgc   1080
cgcgccgccg tccccttctc cctctccagc ctcggggctg tccgcggggg gacggctgcc   1140
ttcggggggg acgggcagg gcggggttcg gcttctggcg tgtgaccggc ggctctagag    1200
cctctgctaa ccatgttcat gccttcttct ttttcctaca gctcctgggc aacgtgctgg   1260
ttattgtgct gtctcatcat tttggcaaag aattc                             1295

<210> 26
<211> 1278
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 26
tcgaggtgag ccccacgttc tgcttcactc tccccatctc cccccctcc ccaccccaa     60
ttttgtattt atttatttt taattattt gtgcagcgat ggggcgggg ggggggggg     120
cgcgcgccag gcggggcggg gcggggcgag gggcgaggcg gagaggtgcg              180
gcggcagcca atcagagcgg cgcgctccga aagtttcctt ttatggcgag gcggcggcgg   240
cggcggccct ataaaaagcg aagcgcgcgg cgggcggcgg gagtcgctgc gttgccttcgccc 300
cgtgccccgc tccgcgccgc ctcgcgccgc ccgccccggc tctgactgac cgcgttactc   360
ccacaggtga gcgggcggga cggcccttct cctccgggct gtaattagcg cttggtttaa   420
tgacggcttcg tttcttttct gtggctgcgt gaaagcctta aagggctccg ggagggccct   480
ttgtgcgggg gggagcggct cggggggtgc gtgcgtgtgt gtgtgcgtgg ggagcgccgc   540
gtgcggcccg cgctgcccgg cggctgtgag cgctgcgggc gcggcgcggg gctttgtgcg   600
ctccgcgtgt gcgcgagggg agcgcggccg ggggcggtgc ccgcggtgc ggggggctg    660
cgaggggaac aaaggctgcg tgcggggtgt gtgcgtgggg gggtgagcag ggggtgtggg   720
cgcggcggtc gggctgtaac cccccctgc accccctcc ccgagttgct gcgcacggcc     780
cggcttcggg tgcggggctc cgtgcgggc gtggcgcggg gctcgccgtg ccgggcgggg    840
ggtggcggca ggtggggtg ccgggcgggg cggggccgcc tcgggccggg gagggctcgg    900
gggagggcg cggcggcccc ggagcgccgg cggctgtcga ggcgcggcga ccgcagcca    960
ttgcctttta tggtaatcgt gcgagagggc gcaggggactt cctttgtccc aaatctggcg  1020
gagccgaaat ctgggaggcg ccgccgcacc ccctctagcg ggcgcgggcg aagcggtgcg   1080
gcgccgcag gaaggaaatg ggcggggagg gccttcgtgc gtcgccgcgc gccgtccccc   1140
ttctccatct ccagcctcgg ggctgccgca ggggacggc tgccttcggg ggggacgggg   1200
cagggcgggg ttcggcttct ggcgttgtac cggcggggtt tatatcttcc cttctctgtt   1260
cctccgcagc cagccatg                                                1278

<210> 27
<211> 229
```

```
<212> DNA
<213> Artificial Sequence


<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 27
gtattagtca tcgctattac catggtgatg cggttttggc agtacatcaa tgggcgtgga    60
tagcggtttg actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg   120
ttttggcacc aaaatcaacg ggactttcca aaatgtcgta acaactccgc cccattgacg   180
caaatgggcg gtaggcgtgt acggtgggag gtctatataa gcagagctc               229


<210> 28
<211> 281
<212> DNA
<213> Artificial Sequence


<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 28
tggcattatg cccagtacat gaccttatgg gactttccta cttggcagta catctacgta    60
ttagtcatcg ctattaccat ggtgatgcgg ttttggcagt acatcaatgg gcgtggatag   120
cggtttgact cacggggatt tccaagtctc caccccattg acgtcaatgg gagtttgttt   180
tggcaccaaa atcaacggga ctttccaaaa tgtcgtaaca actccgcccc attgacgcaa   240
atgggcggta ggcgtgtacg gtgggaggtc tatataagca g                       281


<210> 29
<211> 282
<212> DNA
<213> Artificial Sequence


<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 29
attatgccca gtacatgacc ttatgggact ttcctacttg gcagtacatc tacgtattag    60
tcatcgctat taccatggtg atgcggtttt ggcagtacat caatgggcgt ggatagcggt   120
ttgactcacg gggatttcca agtctccacc ccattgacgt caatgggagt ttgttttggc   180
accaaaatca cgggacttt ccaaaatgtc gtaacaactc cgcccattg acgcaaatgg     240
gcggtaggcg tgtacggtgg gaggtctata taagcagagc tc                      282


<210> 30
<211> 512
<212> DNA
<213> Artificial Sequence


<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 30
ttgcgttaca taacttacgg taaatggccc gcctggctga ccgcccaacg accccgccc     60
attgacgtca ataatgacgt atgttcccat agtaacgcca atagggactt ccattgacg   120
tcaatgggtg gactatttac ggtaaactgc ccacttggca gtacatcaag tgtatcatat  180
```

```
gccaagtacg ccccctattg acgtcaatga cggtaaatgg cccgcctggc attatgccca    240
gtacatgacc ttatgggact ttcctacttg gcagtacatc tacgtattag tcatcgctat    300
taccatggtg atgcggtttt ggcagtacat caatgggcgt ggatagcggt ttgactcacg    360
gggatttcca agtctccacc ccattgacgt caatgggagt ttgttttggc accaaaatca    420
acgggacttt ccaaaatgtc gtaacaactc cgccccattg acgcaaatgg gcggtaggcg    480
tgtacggtgg gaggtctata taagcagagc tc                                  512
```

<210> 31
<211> 308
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
     Synthetic Construct

<400> 31
```
tcggcgaagc ctcgcgcggc cggccaggac gaggagcgcc actaggttga acatccgcac     60
gagccgccgg gccaggtctc ggacgggctc tcgagactcg atctcgtgca tgtcggcggt    120
ccgcggtgag gttatagacc atctgctagg cgggtccggg gagacaggca cattactggc    180
ctcggcgccc agcctaggcg tgtctagagc tcgaccgcgc gtccggagcg ccattcgacc    240
ggcgggtagc gagaagaacg ccggagaccg caggttataa caacgtcatg cataaattaa    300
gaatgggc                                                            308
```

<210> 32
<211> 1848
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
     Synthetic Construct

<400> 32
```
ctgcagtgaa taataaaatg tgtgtttgtc cgaaatacgc gtttgagatt tctgtcccga     60
ctaaattcat gtcgcgcgat agtggtgttt atcgccgata gagatggcga tattggaaaa    120
atcgatattt gaaaatatgg catattgaaa atgtcgccga tgtgagtttc tgtgtaactg    180
atatcgccat ttttccaaaa gttgattttt gggcatacgc gatatctggc gatacgctta    240
tatcgtttac gggggatggc gatagacgcc tttggtgact tgggcgattc tgtgtgtcgc    300
aaatatcgca gtttcgatat aggtgacaga cgatatgagg ctatatcgcc gatagaggcg    360
acatcaagct ggcacatggc caatgcatat cgatctatac attgaatcaa tattggccat    420
tagccatatt attcattggt tatatagcat aaatcaatat tggctattgg ccattgcata    480
cgttgtatcc atatcataat atgtacattt atattggctc atgtccaaca ttaccgccat    540
gttgacattg attattgact agttattaat agtaatcaat tacggggtca ttagttcata    600
gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgcct ggctgaccgc    660
ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta acgccaatag    720
ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac ttggcagtac    780
atcaagtgta tcatatgcca agtacgcccc ctattgacgt caatgacggt aaatggcccg    840
cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag tacatctacg    900
tattagtcat cgctattacc atggtgatgc ggttttggca gtacatcaat gggcgtggat    960
agcggtttga ctcacgggga tttccaagtc tccaccccat tgacgtcaat gggagtttgt    1020
tttggcacca aaatcaacgg gactttccaa aatgtcgtaa caactccgcc ccattgacgc    1080
aaatgggcgg taggcgtgta cggtgggagg tctatataag cagagctcgt ttagtgaacc    1140
gtcagatcgc ctggagacgc catccacgct gttttgacct ccatagaaga caccgggacc    1200
gatccagcct ccgcggccgg gaacggtgca ttggaacgcg gattccccgt gccaagagtg    1260
acgtaagtac gcctataga gtctataggc ccaccccctt ggcttcttat gcatgctata    1320
ctgttttggg cttggggtct atacaccccc gcttcctcat gttataggtg atggtatagc    1380
ttagcctata ggtgtgggt attgaccatt attgaccact cccctattgg tgacgatact    1440
```

```
ttccattact aatccataac atggctcttt gcacaactct ctttattggc tatatgccaa    1500
tacactgtcc ttcagagact gacacggact ctgtattttt acaggatggg gtctcattta    1560
ttatttacaa attcacatat acaacaccac cgtccccagt gcccgcagtt tttattaaac    1620
ataacgtggg atctccagcg aatctcgggt acgtgttccg gacatggggc tcttctccgg    1680
tagcggcgga gcttctacat ccagccctgc tcccatcctc ccactcatgg tcctcggcag    1740
ctccttgctc ctaacagtgg aggccagact taggcacagc acgatgccca ccaccaccag    1800
tgtgcccaca aggccgtggc ggtagggtat gtgtctgaaa atgagctc                 1848
```

<210> 33
<211> 1176
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 33

```
cccgggccca gcaccccaag gcggccaacg ccaaaactct ccctcctcct cttcctcaat     60
ctcgctctcg ctcttttttt ttttcgcaaa aggagggggag aggggtaaa aaaatgctgc    120
actgtgcggc gaagccggtg agtgagcggc gcggggccaa tcagcgtgcg ccgttccgaa    180
agttgccttt tatggctcga gcggccgcgg cggcgcccta taaaacccag cggcgcgacg    240
cgccaccacc gccgagaccg cgtccgcccc gcgagcacag agcctcgcct ttgccgatcc    300
gccgcccgtc cacacccgcc gccaggtaag cccggccagc cgaccggggc atgcggccgc    360
ggcccctttcg cccgtgcaga gccgccgtct gggccgcagc ggggggcgca tggggggga    420
accggaccgc cgtgggggggc gcgggagaag ccctgggcc tccggagatg ggggacaccc    480
cacgccagtt cggaggcgcg aggccgcgct cgggaggcgc gctccggggg tgccgctctc    540
ggggcggggg caaccggcgg ggtctttgtc tgagccgggc tcttgccaat gggatcgca    600
gggtgggcgc ggcgtagccc ccgccaggcc cggtggggc tggggcgcca ttgccggtgc    660
gcgctggtcc tttgggcgct aactgcgtgc gcgctgggaa ttggcgctaa ttgcgcgtgc    720
gcgctgggac tcaaggcgct aattgcgcgt gcgttctggg gccgggggtg ccgcggcctg    780
ggctgggggcg aaggcgggct cggccggaag gggtggggtc gccgcggctc ccgggcgctt    840
gcgcgcactt cctgcccgag ccgctggccg cccgagggtg tggccgctgc gtgcgcgcgc    900
gccgacccgg cgctgtttga accgggcgga ggcggggctg gcgcccggtt gggaggggt    960
tgggcctgg cttcctgccg cgcgccgcgg ggacgcctcc gaccagtgtt tgccttttat   1020
ggtaataacg cggccggccc ggcttccttt gtccccaatc tgggcgcgcg ccggcgcccc   1080
ctggcggcct aaggactcgg cgcgccggaa gtggccaggg cggggggcgac ttcggctcac   1140
agcgcgcccg gctattctcg cagctcacca tggatg                             1176
```

<210> 34
<211> 49
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 34

```
cttctggcgt gtgaccggcg gggtttatat cttcccttcc caagcttgg                  49
```

<210> 35
<211> 66
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

```
<400> 35
cttctggcgt gtgaccggcg gggtttatat cttcccttct ctgttcctcc gcagccccaa        60
gcttgg                                                                    66


<210> 36
<211> 68
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 36
cttctggcgt gtgaccggcg gggtttatat cttcccttct ctgttcctcc gcagccagcc        60
aagcttgg                                                                  68


<210> 37
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 37
cttctggcgt gtgaccggcg gggtttatat cttcccttct ctgttcctcc gcagccagcc        60
atggatgat                                                                 69


<210> 38
<211> 1278
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 38
tcgaggtgag ccccacgttc tgcttcactc tccccatctc cccccctcc ccacccccaa         60
ttttgtattt atttattttt taattatttt gtgcagcgat ggggcgggg ggggggggg        120
cgcgcgccag gcgggcgggg gcggggcgag gggcggggcg gggcgaggcg gagaggtgcg       180
gcggcagcca atcagagcgg cgcgctccga aagtttcctt ttatggcgag gcggcggcgg       240
cggcggccct ataaaaagcg aagcgcgcgg cgggcgggag tcgctgcgtt gccttcgccc       300
cgtgccccgc tccgcgccgc ctcgcgccgc ccgccccggc tctgactgac cgcgttactc       360
ccacaggtga gcgggcggga cggcccttct cctccgggct gtaattagcg cttggtttaa       420
tgacggctcg tttcttttct gtggctgcgt gaaagcctta aagggctccg ggagggccct       480
ttgtgcgggg gggagcggct cggggggtgc gtgcgtgtgt gtgtgcgtgg ggagcgccgc       540
gtgcggcccg cgctgcccgg cggctgtgag cgctgcgggc gcggcgcggg gctttgtgcg       600
ctccgcgtgt gcgcgagggg agcgcggccg ggggcggtgc cccgcggtgc ggggggggctg       660
cgaggggaac aaaggctgcg tgcggggtgt gtgcgtgggg gggtgagcag ggggtgtggg       720
cgcggcggtc gggctgtaac ccccccctgc accccctcc ccgagttgct gcgcacggcc       780
cggcttcggg tgcggggctc cgtgcggggc gtggcgcggg gctcgccgtg ccgggcgggg       840
ggtggcggca ggtggggggtg ccgggcgggg cggggccgcc tcgggccggg gagggctcgg      900
gggaggggcg cggcggcccc ggagcgccgg cggctgtcga ggcgcggcga ccgcagcca        960
ttgcctttta tggtaatcgt gcgagagggc gcagggactt cctttgtccc aaatctggcg      1020
gagccgaaat ctgggaggcg ccgccgcacc ccctctagcg ggcgcgggcg aagcggtgcg     1080
```

```
gcgccggcag gaaggaaatg ggcggggagg gccttcgtgc gtcgccgcgc cgccgtcccc    1140
ttctccatct ccagcctcgg ggctgccgca gggggacggc tgccttcggg ggggacgggg    1200
cagggcgggg ttcggcttct ggcgttgtac cggcggggtt tatatcttcc cttctctgtt    1260
cctccgcagc cagccatg                                                  1278
```

```
<210> 39
<211> 1176
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 39
cccgggccca gcaccccaag gcggccaacg ccaaaactct ccctcctcct cttcctcaat     60
ctcgctctcg ctcttttttt ttttcgcaaa aggaggggag aggggtaaa aaaatgctgc     120
actgtgcggc gaagccggtg agtgagcggc gcgggccaa tcagcgtgcg ccgttccgaa     180
agttgccttt tatggctcga gcggccgcgg cggcgcccta taaaacccag cggcgcgacg     240
cgccaccacc gccgagaccg cgtccgcccc gcgagcacag agcctcgcct ttgccgatcc     300
gccgcccgtc cacacccgcc gccaggtaag cccggccagc cgaccggggc atgcggccgc     360
ggccccttcg cccgtgcaga gccgccgtct gggccgcagc ggggggcgca tggggggggga     420
accggaccgc cgtgggggc gcgggagaag cccctgggcc tccggagatg ggggacaccc     480
cacgccagtt cggaggcgcg aggccgcgct cgggaggcgc gctccggggg tgccgctctc     540
ggggcggggg caaccggcgg ggtctttgtc tgagccgggc tcttgccaat ggggatcgca     600
gggtgggcgc ggcgtagccc ccgccaggcc cggtgggggc tggggcgcca ttgccggtgc     660
gcgctggtcc tttgggcgct aactgcgtgc gcgctgggaa ttggcgctaa ttgcgcgtgc     720
gcgctgggac tcaaggcgct aattgcgcgt gcgttctggg gcccgggggtg ccgcggcctg     780
ggctggggcg aaggcgggct cggccggaag gggtggggtc gccgcggctc ccgggcgctt     840
gcgcgcactt cctgcccgag ccgctggccg cccgagggtg tggccgctgc gtgcgcgcgc     900
gccgacccgg cgctgtttga accgggcgga ggcggggctg gcgcccggtt gggaggggggt     960
tggggcctgg cttcctgccg cgcgccgcgg ggacgcctcc gaccagtgtt tgcctttat    1020
ggtaataacg cggccggccc ggcttccttt gtccccaatc tgggcgcgcg ccggcgcccc    1080
ctggcggcct aaggactcgg cgcgccggaa gtggccaggg cggggggcgac ttcggctcac    1140
agcgcgcccg gctattctcg cagctcacca tggatg                             1176
```

```
<210> 40
<211> 1345
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 40
tcgaggtgag ccccacgttc tgcttcactc tccccatctc cccccctcc ccacccccaa     60
ttttgtattt atttattttt taattatttt gtgcagcgat ggggcgggg gggggggggg    120
cgcgcgccag gcggggcggg gcggggcgag gggcggggcg gggcgaggcg gagaggtgcg    180
gcggcagcca atcagagcgg cgcgctccga aagtttcctt ttatggcgag gcggcggcgg    240
cggcggccct ataaaaagcg aagcgcgcgg cgggcgggag tcgctgcgtt gccttcgccc    300
cgtgccccgc tccgcgccgc ctcgcgccgc ccgccccggc tctgactgac cgcgttactc    360
ccacaggtga gcgggcggga cggcccttct cctccgggct gtaattagcg cttggtttaa    420
tgacggctcg tttctttct gtggctgcgt gaaagcctta aagggctccg ggagggccct     480
ttgtgcgggg gggagcggct cgggggggtgc gtgcgtgtgt gtgtgcgtgg ggagcgccgc    540
gtgcggcccg cgctgcccgg cggctgtgag cgctgcgggc gcggcgcggg gctttgtgcg    600
ctccgcgtgt gcgcgagggg agcgcggccg ggggcggtgc cccgcggtgc ggggggggctg    660
cgaggggaac aaaggctgcg tgcggggtgt gtgcgtgggg gggtgagcag ggggtgtggg    720
cgcggcggtc gggctgtaac cccccctgc acccccctcc ccgagttgct gagcacggcc    780
```

```
cggcttcggg tgcggggctc cgtgcggggc gtggcgcggg gctcgccgtg ccgggcgggg         840
ggtggcggca ggtgggggtg ccgggcgggg cggggccgcc tcgggccggg gagggctcgg         900
gggaggggcg cggcggcccc ggagcgccgg cggctgtcga ggcgcggcga gccgcagcca         960
ttgcctttta tggtaatcgt gcgagagggc gcagggactt cctttgtccc aaatctggcg        1020
gagccgaaat ctgggaggcg ccgccgcacc ccctctagcg ggcgcggggc aagcggtgcg        1080
gcgccggcag gaaggaaatg ggcgggggagg gccttcgtgc gtcgccgcgc cgccgtccccc      1140
ttctccatct ccagcctcgg ggctgccgca gggggacggc tgccttcggg ggggacgggg        1200
cagggcgggg ttcggcttct ggcgtgtgac cggcggctct agagcctctg ctaaccatgt        1260
tcatgccttc ttcttttcc tacagctcct gggcaacgtg ctggttgttg tgctgtctca         1320
tcattttggc aaagaattca agctt                                              1345
```

<210> 41
<211> 684
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
      Synthetic Construct

<400> 41
```
tcaatattgg ccattagcca tattattcat tggttatata gcataaatca atattggcta          60
ttggccattg catacgttgt atctatatca taatatgtac atttatattg gctcatgtcc         120
aatatgaccg ccatgttggc attgattatt gactagttat taatagtaat caattacggg         180
gtcattagtt catagcccat atatggagtt ccgcgttaca taacttacgg taaatggccc         240
gcctggctga ccgcccaacg accccgccc attgacgtca ataatgacgt atgttcccat          300
agtaacgcca atagggactt tccattgacg tcaatgggtg gagtatttac ggtaaactgc         360
ccacttggca gtacatcaag tgtatcatat gccaagtccg cccctattg acgtcaatga        420
cggtaaatgg cccgcctggc attatgccca gtacatgacc ttacgggact ttcctacttg        480
gcagtacatc tacgtattag tcatcgctat taccatggtg atgcggtttt ggcagtacac        540
caatgggcgt ggatagcggt ttgactcacg gggatttcca agtctccacc ccattgacgt         600
caatgggagt ttgttttggc accaaaatca acgggacttt ccaaaatgtc gtaataaccc         660
cgccccgttg acgcaaatgg gcgg                                               684
```